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Accuracy of loop-mediated isothermal amplification for the diagnosis of *Clostridium difficile* infection: a systematic review

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ABSTRACT

Loop-mediated isothermal DNA amplification (LAMP) is currently used as standalone diagnostic test for *C. difficile* infection (CDI). We assessed the diagnostic accuracy of LAMP for the diagnosis of CDI. We searched 5 databases to identify studies that compared LAMP with culture cytotoxicity neutralization assay or anaerobic toxigenic culture (TC) of *C. difficile*. We used the random-effects model to calculate pooled sensitivities, specificities, diagnostic odds ratios, and their 95% confidence intervals (CIs). The search of the databases yielded 16 studies (6979 samples) that met inclusion criteria. When TC was used as the gold standard (6572 samples), bivariate analysis yielded a mean sensitivity of 0.95 (95% CI, 0.93–0.97; $I^2 = 67.4$) and a mean specificity of 0.99 (95% CI, 0.96–1.00; $I^2 = 97.0$). LAMP is a useful diagnostic tool with high sensitivity and specificity for detecting CDI. The results should, however, be interpreted only in the presence of clinical suspicion and symptoms of CDI.

1. Introduction

Clostridium difficile is the most common bacterial cause of inhospital healthcare-associated diarrhea in North America and Europe with an estimated incidence of 3.85 cases per 1000 patient-days in US acute care hospitals (Zimlichman et al., 2013). Over the last several years, new, highly virulent strains, such as BI-NAP1-027, have caused several global outbreaks (Loo et al., 2005). These strains have been shown to cause more severe disease than the non-NAP1 strains and are associated with high level fluoroquinolone resistance (Deshpande et al., 2008; Loo et al., 2005; McDonald et al., 2005). In addition to the associated morbidity and mortality, the cost of hospital-acquired *C. difficile* infections (CDIs) has been estimated to exceed $1.5 billion a year in the United States (Zimlichman et al., 2013). Nearly all antimicrobials have been associated with CDI over the years, with longer exposure and exposure to multiple antimicrobial agents increasing the risk of CDI further (Pepin et al., 2005). The diagnosis of CDI is usually made based on the presence of symptoms (diarrhea, abdominal pain, and fever) and either a stool test result positive for *C. difficile* toxins or toxigenic *C. difficile* or colonoscopic findings demonstrating pseudomembranous colitis (Cohen et al., 2010). Laboratory test methods available for identification of CDI include anaerobic toxigenic culture (TC), culture cytotoxicity neutralization assay (CCNA), enzyme immunoassay (EIA) for toxin A and B, glutamate dehydrogenase (GDH) EIA, real-time polymerase chain reaction (RT-PCR), and loop-mediated isothermal amplification assay (LAMP) (O'Horo et al., 2012). There is currently no accepted gold standard test for diagnosis of CDI, but CCNA (sensitivity of 70–100%, specificity of 90–100%) and anaerobic TC (sensitivity of 90–100%, specificity of 98–100%) are often used as reference tests for evaluation of each other as well as evaluation of novel testing methods (Massey et al., 2003; Poutanen and Simor, 2004). CCNA and anaerobic TC are time-consuming and resource-intensive tests. In practice, many labs perform the rapid and easy-to-perform EIA for toxin A and B detection, though this test lacks sensitivity and is considered a suboptimal approach by current clinical practice guidelines (Cohen et al., 2010). More recently, nucleic acid amplification tests including RT-PCR and LAMP have been developed for diagnosis of CDI. Some hospitals have already begun to implement these tests in order to improve the rapidity of

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CDI testing and detection rates. Two previous meta-analyses have evaluated at the diagnostic characteristics of RT-PCR. They found that while it is highly sensitive and specific, it is also dependent on CDI prevalence (Deshpande et al., 2011; O'Horo et al., 2012). While most commercially available RT-PCR assays are designed to detect a conservative region of tcdB, it has been reported that in *C. difficile* variant strains, tcdA is more conserved (Rupnik, 2008). The Illumigene™ *C. difficile* Assay (Meridian Bioscience, Cincinnati, OH, USA) uses loop-mediated isothermal amplification technology to detect a 204-bp sequence in the conserved 5′ region of tcdA. While some variant *C. difficile* strains have deletions at the 3′ end of the tcdA gene, the 5′ portion remains intact for these strains (Rupnik et al., 1998). A recent study confirmed the ability of the Illumigene assay to detect these Toxin A−/B+ strains (Couturier et al., 2013). The Illumigene assay is currently the only commercial LAMP assay approved for US laboratory use. The Food and Drug Administration now requires all vendors to include toxigenic culture as a comparator for any new diagnostic test for *C. difficile* detection. One systematic review evaluated the diagnostic capabilities of LAMP for detection of CDI and concluded that LAMP was a promising test, but further investigation was necessary to evaluate LAMP as a diagnostic tool (O'Horo et al., 2012). The most recent Infectious Diseases Society of America (IDSA)/Society for Healthcare of America (SHEA) guidelines on diagnostic testing of *C. difficile* suggest that more data are needed on nucleic acid amplification tests before it can be implemented for routine use (Cohen et al., 2010). We performed a meta-analysis to assess the capabilities of LAMP in the diagnosis of CDI. The aim of this study was to investigate whether LAMP is sensitive and specific enough for the diagnosis of CDI when used as a standalone test.

2. Methods

This review was performed with a standardized written protocol that followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement guidelines (Moher et al., 2009).

2.1. Search strategy

We systematically searched the literature using the following predetermined inclusion criteria. Studies evaluating LAMP as a diagnostic test for CDI were eligible for inclusion if the studies 1) described original research; 2) performed stool specimen analyses from inpatients or outpatients; 3) compared LAMP to a reference method – either CCNA or anaerobic TC; 4) reported total number of patients tested and positive/negative results that allow calculation of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN); and 5) in any language. We excluded studies if 1) all samples were not tested by at least 1 reference test, that is, CCNA or anaerobic TC; 2) if the reference test was performed only on a subset of samples, that is, only positives, negatives, or those that were discordant; 3) the reference test was a combination of >1 diagnostic test; and 4) they involved animal studies or laboratory cultures of *C. difficile*. In the excluded studies, LAMP tests were confirmed using other diagnostic tests but not CCNA or anaerobic TC. We also excluded combination reference tests because we wanted to individually calculate the diagnostic accuracy of LAMP vis-a-vis the 2 most accepted reference standards. This search was performed in August 2014. The following databases were searched since their inception: PubMed, Web of Science, Embase, Cochrane Library, and Scopus. In addition, we reviewed the reference lists of several previously published reviews on *C. difficile* diagnostic testing. Search terms included "*Clostridium difficile*, *C. difficile*, CDI, sensitivity, specificity, screening, false positives, false negatives, accuracy, predictive value, diagnostic test, diagnosis." Reference lists from included studies were also searched. In addition, experts and commercial LAMP assay manufacturer for *C. difficile* diagnosis were contacted for additional studies. The electronic search strategy of PubMed is available in Supplementary Appendix 1.

2.2. Study selection

A list of retrieved articles was reviewed independently by 2 investigators (A. L. and V. P.) in order to choose potentially relevant articles, and disagreements about particular studies were discussed and resolved. When multiple articles for a single study had been published by the same authors, we used the most relevant publication and supplemented it, if necessary, with data from other publications. Authors of studies were contacted when the information was not available in the published study.

2.3. Data extraction

Two investigators (A. L. and V. P.) independently extracted data from full text of the included studies, and disagreements were discussed and resolved. All studies evaluated the diagnostic accuracy of LAMP on a per-sample basis.

2.4. Assessment of study quality

The methodological quality for each paper was assessed independently by 2 investigators (A. L. and V. P.) using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (Whiting et al., 2011) criteria, and disagreements were discussed and resolved.

2.5. Data synthesis and meta-analysis

The TP, FP, TN, and FN were taken directly from the source papers. Where this information was not available, the values were calculated from the data that were provided in the article. In some cases, the corresponding authors of the article were contacted to gather information for creating the 2×2 table. Data were analyzed using the 'midas' and 'metandi' module for STATA (version 12) and 2 freeware programs, i.e., Open Meta[analyst] (version 1.4) (Wallace et al., 2012) and Metadisc (MetaDiSc version 1.4). To synthesize data, we employed a bivariate random-effects model. The bivariate model pairs sensitivity and specificity simultaneously for each study, accommodating their natural correlation, thus preserving the 2-dimensional nature of the data and allows for negative correlation within studies (Reitsma et al., 2005). Using a random-effects approach for both sensitivity and specificity, the model also accounts for clinical and methodological heterogeneity between the studies (DerSimonian and Laird, 1986). We also calculated the positive likelihood ratio (PLR), negative likelihood ratio (NLR), and a hierarchical summary receiver operating characteristics (HSROC) curve. To address 0 observations in 2×2 contingency tables, continuity correction was achieved by adding 0.5 to each cell. We calculated κ statistics to assess the agreement between the 2 investigators for study selection, data extraction, and assessment of methodological quality.

2.6. Investigations of heterogeneity

To assess heterogeneity, we calculated the inconsistency index, I^2. An I^2 of 33–66% was considered as moderate heterogeneity. To address potential heterogeneity among studies, we performed subgroup analysis on prespecified variable: the calculated prevalence of *C. difficile* (<15% and ≥15%). As different cut-offs or thresholds were not expected for the LAMP assay among the studies, we did not explore threshold effect as a potential source of heterogeneity.

2.7. Publication bias

The presence and effect of publication bias were examined using Deeks' regression test of asymmetry (Deeks et al., 2005) and Egger's test (Egger et al., 1997).

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