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RESEARCH **ORIGINAL ARTICLE**

Improving Pediatric Clostridioides Difficile Testing Practices: Implementation of a 2-Tier Diagnostic Pathway

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ABSTRACT

INTRODUCTION - *Clostridioides difficile (C. difficile)* is a pathogen of significant concern for children, especially those hospitalized or with underlying medical conditions. Diagnosis of *C. difficile* infection (CDI) in these patients can be challenging due to asymptomatic colonization. Inappropriate testing and non-adherence to laboratory testing guidelines can result in increased false-positive, rates precipitating unnecessary isolation precautions and antibiotic treatment for such patients.

METHODS - This retrospective cohort study aimed to evaluate the effectiveness of a new diagnostic pathway for *C. difficile* testing that was implemented in two pediatric hospitals. The study design collected data over two years, starting one year before and ending one year after pathway implementation. The study highlighted the importance of appropriate testing and the need for interventions to improve testing practices in pediatric patients. A 2-tier testing algorithm was implemented, consisting of polymerase chain reaction (PCR) for the presence or absence of the toxin B gene and an enzyme immunoassay for toxin A/B production. The best practice advisory was used to determine when *C. difficile* testing should not be performed. The chi-square and Fisher's Exact tests were used for data analysis using SPSS version 29.

RESULTS - The study found a significant association between the implementation of the *C. difficile* testing pathway and the test positivity rates for both inpatient and emergency department (ED) patients at both hospitals. Out of 159,434 inpatients in Hospital A, 71 had positive *C. difficile* test results, and out of 11,109 inpatients in Hospital B, nine had positive test results. Similarly, out of 121,951 ED patients in Hospital A, eight had positive test results, and out of 67,999 ED patients in Hospital B, 16 had positive test results. There was a statistically significant association between the pre and post-pathway implementation years for both hospitals ($p<0.001$ for Hospital B inpatient and ED, $p=0.033$ for Hospital A inpatient, and $p=0.004$ for Hospital A ED).

CONCLUSION - Adherence to laboratory testing guidelines including appropriate testing based on factors such as the patient's age, underlying health conditions, recent antibiotic use, and the presence of other infections or illnesses can reduce unnecessary testing and false-positive rates. False-positive results can occur in pediatric patients due to the high rate of asymptomatic colonization, making it essential to use a combination of clinical symptoms, history, and appropriate diagnostic testing to minimize the risk of misdiagnosis.

KEY WORDS- Clostridioides difficile, Clinical Pathway, Pediatrics, polymerase chain reaction, Diagnosis

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INTRODUCTION

Clostridioides difficile, also known as *C. difficile*, is a leading cause of healthcare-associated diarrheal illness in adults in the United States. However, it is increasingly being recognized as a significant problem in children, especially those who are hospitalized or have underlying medical conditions [1]. Accurate diagnosis of *C. difficile* infection (CDI) in pediatric patients can be challenging, as asymptomatic colonization is common, particularly in neonates and infants [2]. Asymptomatic carriage can lead to false-positive test results, making it difficult to differentiate between colonization and infection. Appropriate use of testing and adherence to laboratory testing guidelines are crucial to reduce unnecessary testing and false-positive results [3,4].

Although several testing modalities are available, nucleic acid amplification testing (NAAT) and enzyme immunoassay (EIA) are most commonly utilized [5]. Clinical practice guidelines recommend a multi-step algorithm rather than using NAAT alone for diagnosing CDI [5,6]. Healthcare providers should rely on a combination of clinical symptoms, patient history, and appropriate diagnostic testing to minimize the risk of misdiagnosis.

In this study, the authors evaluated the effect of implementing a pathway and best practice advisory on the positivity rate of *C. difficile* among inpatient units and the emergency department at their institution. The aim was to reduce unnecessary testing and false-positive rates. The study highlights the importance of appropriate testing and the need for interventions to improve testing practices in pediatric patients.

METHODS

STUDY DESIGN - This project was conducted in two hospitals, Hospital A and Hospital B. This study aimed to evaluate the effectiveness of a newly implemented diagnostic pathway for *C. difficile* testing. The study design was a retrospective cohort study in which data was collected over two years, starting one year before until one year after the pathway implementation.

DIAGNOSTIC PATHWAY - In collaboration with the antimicrobial stewardship and infection prevention and control committees, a 2-tier *C. difficile* testing algorithm was implemented. All patient stool samples were initially tested using Xpert *C. difficile* (Cepheid, Sunnyvale, CA), a real-time polymerase chain reaction (PCR) test that determined the presence or absence of the toxin B gene (tcdB). All PCR-positive patient stool samples were subsequently tested by C. diff Quik Chek Complete (Alere, Orlando, FL), an enzyme immunoassay that determined the presence or absence of active Toxin A/B production.

SPECIMEN REQUIREMENTS - There were no changes in specimen collection and handling requirements. Fresh, liquid stool was collected in a sterile container and sent to the laboratory. All testing was performed within the test manufacturer's recommended timelines. *C. difficile* testing was not performed on solid stool, or any stool samples placed in liquid transport media (i.e., Cary-Blair).

SITUATIONS WHERE TESTING WAS NOT PERFORMED- Best Practice Advisory (BPA) alerts were implemented in the electronic health record (Epic, Verona, WI) at the time of provider order entry to maximize the pre-test probability of disease and minimize false positive test results. Testing was highly discouraged when: the patient was ≤ 2 years of age (required approval by Infectious Diseases), the patient was producing < 3 liquid stools in a 24-hour period, the patient had received laxatives in the previous 48 hours, the patient's stool sample did NOT conform to the shape of the collection container, the patient had a negative *C. difficile* test within the last 7 days, or the patient had a positive *C. difficile* test within the last 14 days.

DATA ANALYSIS - Data was collected over two years, starting one year before until one year after the implementation of the pathway. The chi-square and Fisher's Exact tests were used to analyze the data using SPSS version 29 (IBM Corporation, Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant. The study was determined not to be human subject research by our institutional review board (274710).

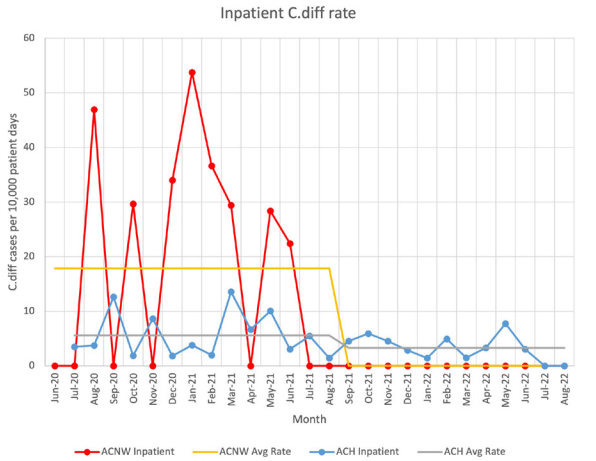
RESULTS

For Hospital A inpatients, out of 159,434 patients, seventy-one had positive *C. difficile* test results. There was a statistically significant association between Hospital A pre and post-pathway implementation and the test positivity rate ($p = 0.033$). For Hospital B inpatients, out of 11,109 patients, nine had positive *C. difficile* test results. Using the Chi-square test, we found a statistically significant association between Hospital B inpatient pre and post-pathway implementation and the *C. difficile* test positivity rate ($p < 0.001$). (Figure-1).

For Hospital A ED, out of 121,951 patients, eight had positive *C. difficile* test results. There was a

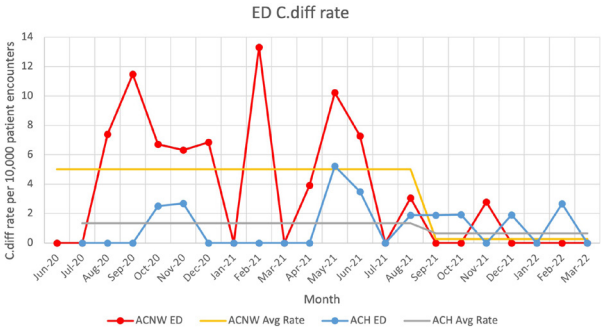
statistically significant association between Hospital A ED pre and post-pathway implementation and the test positivity rate ($p = 0.004$). For Hospital B ED, out of 67,999 patients, sixteen of them had positive *C. difficile* test results. There was a statistically significant association between the satellite hospital ED pre and post-pathway implementation and the *C. difficile* test positivity rate ($p < 0.001$). (Figure-2)

Figure 1. Inpatient C. diff Rate



*Inpatient C.diff rates at both hospitals during the study period. ACNW: Arkansas Children's Northwest, ACH: Arkansas Children's Hospital

Figure 2. Emergency Department C.diff Rate



*Emergency Department C.diff rates at both hospitals during the study period. ED: Emergency Department, ACNW: Arkansas Children's Northwest, ACH: Arkansas Children's Hospital

DISCUSSION

Accurate diagnosis of CDI in pediatric patients requires healthcare providers to use a combination of clinical symptoms in the history and appropriate diagnostic testing to minimize the risk of misdiagnosis [6]. Various testing modalities are available, including nucleic acid amplification testing (NAAT) which typically uses polymerase chain reaction (PCR) to detect genes for toxin A or B; and enzyme immunoassay (EIA) to detect the antigen for glutamate dehydrogenase (GDH) or for toxins A and B [5]. According to the 2017 clinical practice guidelines published by the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA), appropriate use of testing based on factors such as the patient's age, underlying health conditions, recent antibiotic use, and the presence of other infections or illnesses should be practiced [6]. Adherence to laboratory testing guidelines can also reduce unnecessary testing that could lead to an increase in false-positive rates [7].

Neither toxin A nor toxin B were initially tested in the diagnostic pathway. To identify toxin-producing strains of *C. difficile*, the presence of a genetic element called the pathogenicity locus (PaLoc) is examined. This genetic element consists of five genes: tcdA (Toxin A gene), tcdB (Toxin B gene), tcdE (upregulates tcdA and tcdB transcription), tcdC (downregulates tcdA and tcdB transcription), and tcdE (holin gene responsible for creating cell membrane pores, allowing the synthesized toxin to be released into the extracellular environment).

The first step in the diagnostic pathway involves determining the presence or absence of toxin-producing *C. difficile* by testing for one or more genes within the PaLoc. The most reliable genetic target to establish the presence or absence of the PaLoc is the Toxin B gene (tcdB). Our first-tier test, the Cepheid GeneXpert, is used to detect the presence or absence of tcdB. If tcdB is present, the second-tier test is conducted to determine the presence or absence of actual Toxin A or B in the stool sample.

False-positive results can occur in pediatric patients due to the high rate of asymptomatic colonization, which can be detected in up to 33% of children under two years of age [2]. To minimize false-positive results, a two-tiered approach to *C. difficile* testing can be used. Nucleic acid amplification (e.g., PCR) is the preferred first-tier test and should target one or more genetic elements within the pathogenicity locus (PaLoc); the *C. difficile* toxin A (tcdA) or B (tcdB) genes are most commonly utilized [8]. NAAT is highly sensitive and can detect minuscule quantities of *C. difficile* DNA in the stool sample. With this high degree of sensitivity, a negative NAAT test result essentially rules out infection. However, a positive NAAT test cannot determine the presence or absence of active toxin production, which can lead to false-positive results and the treatment of colonized patients [9]. Stool samples positive by NAAT are subsequently assessed for active toxin production using an EIA test that

detects the presence of *C. difficile* toxin A and/or toxin B [10]. EIA is less sensitive than NAAT, but highly specific for detecting active CDI, essentially eliminating false-positive results. The combination of NAAT followed by EIA when applicable is an optimal testing strategy to minimize false positive test results [6]. In addition, this testing strategy is exceptionally sensitive at detecting colonized individuals (e.g., NAAT positive, EIA negative), which is important for the timely implementation of infection prevention and control measures to reduce the spread of this pathogen within the community and healthcare system [4].

CONCLUSION

Early and accurate detection of *C. difficile* is crucial for prompt and effective treatment in pediatric patients. However, accurate diagnosis of CDI in pediatric patients can present a challenge due to the high rate of asymptomatic colonization, which can lead to false-positive results and unnecessary care. Healthcare providers should use a combination of clinical symptoms from the history and appropriate diagnostic testing to minimize the risk of misdiagnosis. Adherence to a diagnostic pathway that includes laboratory testing guidelines and a BPA in your medical record can also reduce unnecessary testing that could lead to an increase in false-positive rates.

DISCLOSURES & DECLARATIONS

All authors indicate that they received no funding for this work, and has no financial or non-financial interests, study received an IRB exemption for the UAMS IRB.

AUTHOR CONTRIBUTIONS

SS;AD;CF;BS made substantial contributions to the conception of the work and the acquisition of data. Drafted the work or revised it critically for important intellectual content, and approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

EM;LS;MDH;BLB revised it critically for important intellectual content, and approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

EB performed all the statistical analysis and critically reviewed and revised the manuscript.

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