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Original Research Article

A survey on *Clostridioides difficile* infection (CDI) testing in microbiology laboratories in Saudi Arabia: Findings and implications

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Abstract

Purpose: *To investigate various critical elements of Clostridioides difficile infection (CDI) testing in laboratories in Saudi Arabia, encompassing diagnostic techniques employed, testing protocols, specimen handling procedures, result reporting practices, and resource availability.*

Method: *A cross-sectional study was conducted via an online survey to assess CDI testing protocols and procedures employed by microbiologists in Saudi Arabia. The questionnaire examined various aspects of testing procedures, laboratory protocols, testing schedules, and obstacles to conducting CDI tests. Differences were compared using Chi-square. $P < 0.05$ was considered significant.*

Results: *The survey elicited responses from 68 hospitals across 13 administrative regions of Saudi Arabia. A total of 52.9 % (n = 36) came from small hospitals (≤ 200 beds), distributed across 8 regions. The Western region contained the highest number of responding hospitals overall (44.1 %, n = 30). There was significant difference in positive CDI tests reported between large hospitals (> 200 beds; 59.4 %, n = 19) and small hospitals (13.9 %, n = 5; $p < 0.001$). Among laboratories that test in-house, 22.7 % (n = 15) reported using nucleic acid amplification tests, 25.8 % (n = 17) reported sending stool specimens to external laboratories, 11.8 % (n = 8) reported using multistep methods, and 25.8 % (n = 17) were unsure of the used tests. Limited institutional budget for CDI testing kits was the most commonly reported barrier by laboratory microbiologists.*

Conclusion: *While CDI testing practices in Saudi Arabian microbiology laboratories generally align with international guidelines, this survey identifies opportunities for improvement through enhanced education, implementation of evidence-based testing algorithms, and addressing resource limitations.*

Keywords: *Clostridioides difficile, Clostridium difficile, Microbiology, Laboratory technique, Saudi Arabia*

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INTRODUCTION

An accurate diagnosis of *Clostridioides difficile* infection (CDI) is important as it is a major cause of healthcare-associated diarrhea, increasing hospital stay, as well as the risk of sepsis, along with an increased need for surgical interventions [1]. However, key challenges with CDI diagnosis need to be addressed since the frequency and severity of CDIs have been increasing globally, resulting in common hospital-acquired infections [2]. The 10-year cumulative incidence of CDIs in Saudi Arabia is 8.4 %, which is a concern [3]. Both symptomatic and asymptomatic carriers colonized with toxigenic strains play a role in CDI epidemiology [4]. Furthermore, differentiating these groups via laboratory testing is necessary to guide treatment and isolation protocols due to increasing concerns with misdiagnosis and its associated implications [5,6].

Standardized evidence-based guidelines for CDI diagnosis are currently lacking in Saudi Arabia despite the availability of robust and evidence-based guidelines from groups such as the American Society of Colon and Rectal Surgeons, Infectious Diseases Society of America and the National Institute for Health and Care Excellence in the UK [2,5]. Instead, testing criteria have been developed across institutions in Saudi Arabia, which typically apply their protocols internally [5]. There is a concern that this heterogeneity may lead to inaccurate diagnosis and inappropriate management, which needs to be addressed [4].

Table 1 summarizes the key diagnostic methods for *Clostridioides difficile* infection (CDI) as recommended by clinical practice guidelines, highlighting the frequency of recommendation, coincidence rates, and the highest level of evidence supporting each method [7,8]. Therefore, this study investigated current practices related to *C. difficile* testing in hospital microbiology laboratories across Saudi Arabia. Elucidating current protocols and perceptions will identify gaps and recommend robust guidelines for such practices in Saudi Arabia. This would therefore improve diagnosis, management, and surveillance of *C. difficile* testing in microbiology laboratories across Saudi Arabia [9].

METHODS

Study design and population

This was a cross-sectional study conducted from November 2021 to July 2022. It focused on all clinical microbiology laboratories in Saudi Arabia capable of conducting CDI testing, encompassing both hospital-based laboratories and independent private clinical ones. The study targeted accredited hospitals by the Central Board for Accreditation of Healthcare Institutions (CBAHI), Saudi Arabia's national accreditation body, with the survey distributed through the Saudi Commission for Health Specialties (SCFHS) to all registered microbiologists across the Kingdom. Electronic distribution through the SCFHS enabled efficient data collection from the target group of microbiologists across Saudi Arabia. Responses from laboratory tests were gathered from personnel in charge of CDI testing protocols at each facility. However, since there was no access to the total number of registered and actively practicing hospital microbiologists to whom the survey link was sent, it was difficult to calculate the response rate in practice.

Questionnaire development

The questionnaire was created by thoroughly reviewing prior research on microbiologists' practices related to diagnosing and testing of CDIs, with specific focus on evaluating laboratory-specific testing protocols. Published studies assessing microbiologists' understanding and real-world application of CDI diagnostic and testing procedures were also reviewed to guide questionnaire development [10]. The study targeted accredited hospitals by the Central Board for Accreditation of Healthcare Institutions (CBAHI), Saudi Arabia's national accreditation body.

Table 1: Summary of diagnostic methods for *Clostridioides difficile* infection (CDI) recommended by clinical practice guidelines

Diagnostic method	Number of guidelines mentioning this	Coincidence rates	Highest level of evidence
Nucleic acid amplification test (NAAT)	11	80-100%	1a
Glutamate dehydrogenase (GDH) test	10	80-100%	1a
loop-mediated isothermal amplification (LAMP)	6	Not specified	Not specified
Enzyme immunoassay (EIA) for toxins A and B	11	Not specified	1a

*Data adapted from published clinical practice guidelines [7,8]

The survey was distributed through the Saudi Commission for Health Specialties (SCFHS) to all registered microbiologists across the Kingdom. The sample size included both governmental/semi-governmental (47.1 %) and private (53 %) healthcare facilities across different regions of Saudi Arabia, with representation from Western (44.1 %), Central (38.2 %), Eastern (14.7 %), and other regions (2.9 %). By examining these earlier evaluations of microbiologists' knowledge and practices concerning CDI diagnosis and testing, the investigators were able to design an evidence-based questionnaire to effectively evaluate this target group.

The questionnaire consisted of two sections. The first section gathered demographic information, which included test laboratory site, year of establishment, service scope, region, hospital size, practice site type, training level, years of experience, type of hospital, and accreditation status. The second section comprised questions assessing participants' self-reported everyday activities concerning the diagnosis of CDI. Assessments were conducted on existing CDI diagnostic methods, including nucleic acid amplification test (NAAT), toxin EIA, and GDH, along with evaluation of testing algorithms and daily/monthly testing volumes for CDI, as well as criteria for accepting and rejecting samples. The survey also collected data on procedures for reporting test results, practices for testing antimicrobial susceptibility, test ordering, protocols for interpreting test results, and quality control measures employed in the laboratories. This subjective assessment included preparedness of the laboratory for *C. difficile* testing, frequency of CDI testing, types of diagnostic tests, and barriers encountered in the CDI testing process. The opening page explained the study aims and estimated duration to complete the questionnaire. It also included informed consent details, and the web-based format allowed streamlined data entry and analysis while maintaining rigorous informed consent procedures.

Data analysis and ethical approval

The survey data were analyzed using Stata 17.0 (Stata Corp LLC, College Station, TX, USA). Calculations were performed for demographic characteristics, including hospital size (number of beds), geographic location, laboratory setting (hospital-based or private), diagnostic methods employed, testing volumes, sample acceptance criteria, and testing protocols/algorithms used in CDI testing practices. Data were presented in frequency and percentages ad compared using Chi-square tests or Fisher's exact test. $P < 0.05$ was considered statistically significant. The total number of accredited hospitals in Saudi Arabia is 424 (https://portal.cbahi.gov.sa/arabic/accreditation-status/s/hh). Assuming one response per hospital to ensure independence of observations, a sample of 59 hospitals would be needed to achieve a confidence level of 90 % and a margin of error of 10 %. However, it is important to note that the actual number of microbiologists may vary per hospital, which could impact the interpretation of results. Approval for the study was obtained from the Regional Research Ethics Committee, Qassim region, Saudi Arabia (approval no. 1443-441172).

RESULTS

Socio-demographic characteristics

A total of 68 respondents from various regions of Saudi Arabia completed the survey. Majority were < 35 years 51.5 % (n = 35), drawn from the Western region (44.1 %, n = 30), and small hospitals (52.9 %, n = 36; Table 2).

Table 2: Socio-demographic characteristics (n = 68)

Characteristic		N (%)
Age (years)	< 35	35(51.5)
	≥ 35	33(48.5)
Region of laboratory	Western region	30(44.1)
	Central region	26(38.2)
	Eastern region	10(14.7)
	Others (Northern/Southern region)	2(2.9)
*Size of hospital (n = 47)	≤ 200 beds	15(31.9)
	> 200 beds	32(68.1)
Level of training	Specialist	43(63.2)
	Consultant**	15(22.1)
	Resident	10(14.7)
Years of experience	< 10 years	39(57.4)
	≥ 10 years	29(42.7)
Type of hospital	Governmental or Semi-governmental hospital	32(47.1)
	Private lab	21(30.9)
	Private hospital	15(22.1)

*Only participants working in hospitals were reported. Participants from private labs were excluded. **Consultant is a higher rank than the specialist

Age classification

The current binary classification (< 10 years and >10 years of experience) was specifically chosen to reflect professional experience levels that

significantly impact laboratory practice competency. This classification provides more meaningful analysis compared to the sample size (n = 68).

Healthcare regions

Data were presented for Western (44.1 %), Central (38.2 %), and Eastern (14.7 %) regions and accounted for 97 % of responses. While Saudi Arabia has multiple healthcare regions, this presentation reflects the actual distribution of CBAHI-accredited facilities with microbiology laboratories participating in the study. The remaining regions (2.9 %) had too few responses to meaningfully represent separately.

Hospital classification

The binary classification (< 200 and > 200 beds) was based on the CBAHI accreditation criteria for laboratory service requirements and the distribution of respondent facilities

Laboratory practices for *Clostridioides difficile* testing

There was a significant difference between large and small hospitals in routinely reporting positive *C. difficile* specimens ($p < 0.001$). There was no significant difference in routine *C. difficile* susceptibility testing between hospital sizes ($p = 1$; Table 3).

Frequency of stool sample testing and positive C. difficile cases by hospital size

There was no significant difference in the frequency of stool specimens between large and small hospitals ($p > 0.05$). While large hospitals reported receiving specimens at least monthly (17/31, 54.8 %), most small hospitals reported receiving specimens less frequently or never (8/16, 50.0 %; Table 4).

C. difficile diagnostic tests used by Saudi Arabian laboratories

The most commonly used test by microbiology laboratories in Saudi Arabia was NAATs, such as polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP), as reported by 22.7 % (n = 15) of the respondents. On the other hand, 25.8 % (n = 17) of laboratories indicated that they send stool samples to an external lab for CDI testing. The same proportion (25.8 %, n = 17) was unsure which CDI tests are used in their laboratory. Older single-step immunoassays like EIAs for glutamate dehydrogenase (GDH) or toxin were used by only 1.5 - 4.6 % (n = 1 - 3) of laboratories. Some laboratories utilize multistep algorithms that apply a positive screening test followed by a confirmatory test for enhanced accuracy (Table 5).

Table 3: *C. difficile* testing practices

Question	> 200 beds	≤ 200 beds	*P*-value
Functional Laboratory Equipment for *C. difficile* testing	19(59.4)	12(33.3)	0.056
Specific Algorithm for *C. difficile* testing	14(43.8)	10(27.8)	0.262
C. difficile testing regardless of medication/history	18(56.3)	15(41.7)	0.327
C. difficile test regardless of stool consistency	11(34.4)	12(33.3)	1
Reporting *C. difficile* positive specimens	19(59.4)	5(13.9)	<0.001
Routine *C. difficile* susceptibility test	1(3.1)	1(2.8)	1

Table 4: Frequency of stool sample testing and positive *C. difficile* cases by hospital size (N, %)

Category	Frequency	> 200 beds (n = 31)	≤ 200 beds (n = 16)	*P*-value
Frequency of receiving stool specimens	Daily	5(71.4)	2(28.6)	0.940
	Weekly	6(75.0)	2(25.0)	
	Monthly	6(60.0)	4(40.0)	
	Every 6 months	4(57.1)	3(42.9)	
	Never received stool specimens	10(66.7)	5(33.3)	
Frequency of positive *C. difficile* specimens every month	<1 day	15(65.2)	8(34.8)	0.345
	<10 every month	8(53.3)	7 (46.7)	
	≥ 10 every month	2(100)	0(0)	

Table 5: Type of CDI testing in the microbiology laboratories (n = 68)

Tests used to diagnose *C. difficile* infection		**N (%)**
Single tests	NAAT only, e.g., PCR or LAMP	15(22.7)
	C. difficile included in a GI panel of multiple pathogens (e.g., Biofire)	4 (6.06)
	EIA	3 (4.55)
	Combined GDH assay and toxin EIA	1 (1.52)
	Toxigenic culture (*C. difficile* culture followed by detection of toxins)	1 (1.52)
Multistep tests	NAAT followed by EIA for toxin (if NAAT positive)	3(4.6)
	GDH EIA followed by cell cytotoxicity neutralization assay or toxin EIA (if GDH positive)	2(3.0)
	GDH EIA followed by NAAT (if GDH positive)	2(3.0)
	Combined GDH/toxin EIA, followed by NAAT for discrepant results	1(1.5)
Other responses	Stool samples are sent to an external lab	17(25.8)
	Not sure	17(25.8)

EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; GI, gastrointestinal; LAMP, loop mediated isothermal amplification; NAAT, nucleic acid amplification test; PCR, polymerase chain reaction

Table 6: Barriers to C. difficile testing reported by surveyed laboratories (n = 68; n, %)

Barrier	**> 200 beds (n=32)**	**≤ 200 beds (n=36)**	***P*-value**
Limited funding	7(21.9)	10(27.8)	0.575
Neglected pathogen in the hospital	4(12.5)	4(11.1)	0.859
Kit not available	6(18.8)	9(25.0)	0.535
CDI testing not requested by physicians	11(34.4)	11(30.6)	0.7368
No barriers	11(34.4)	12(33.3)	0.928

Barriers to *C. difficile* testing reported by laboratories

The most commonly reported barrier to CDI testing were limited funding (21.8 %; n = 7) in larger hospitals and 27.8 % (n = 10) in smaller hospitals; physicians not ordering CDI tests (34.4 %, n = 11) in larger hospitals and 30.6 % (n = 11) in smaller hospitals (Table 6).

DISCUSSION

This is the first national survey to detail current laboratory testing practices for CDI across Saudi Arabian healthcare institutions. As a result, this study offered foundational data regarding current diagnostic techniques and testing procedures that are being utilized in participating laboratories. This builds on an earlier study by Almutairi et al [11] who surveyed the knowledge of CDI diagnostics among healthcare providers. The study found a knowledge gap among healthcare providers regarding the methods and correct diagnostic tests that should be used to diagnose CDI. Limited funding was the most reported barrier for *C. difficile* testing.

The most used diagnostic test reported was NAATs, including PCR (22.7 %), however, about a quarter of laboratories sent specimens externally or were unsure of testing methods. The fact that NAATs were the predominant diagnostic test indicates a preference for highly sensitive detection, albeit with lower specificity compared to multistep algorithms. Furthermore, NAATs directly detect the presence of *C. difficile* bacteria by amplifying their nucleic acid sequences and are considered highly sensitive for CDI diagnosis. However, NAATs have reduced specificity compared to multistep algorithms since they detect both toxigenic and non-toxigenic strains. Despite some common trends, significant differences were also observed with findings from other countries. This study revealed a high prevalence of NAATs (22.7 %), contrasting with the dominance of EIAs in studies from Spain (52.5 %) and England (53 %) [12]. This indicated greater reliance on sensitive molecular tests compared to older immunoassays. In contrast, only 1.5 % of the laboratories reported using GDH plus NAAT combination, indicating missed opportunities to maximize diagnostic accuracy through evidence-based testing protocols, since both have high sensitivity but moderate specificity [7].

An optimal multistep testing method should involve GDH followed by toxin EIA as recommended by major international guidelines [7,8]. The GDH assays detect *C. difficile* more broadly but cannot differentiate toxigenic strains, therefore, toxin EIA is needed to identify whether the detected *C. difficile* is toxigenic. The lower sensitivity of EIA is complemented by the high sensitivity of GDH, whereas the high specificity of EIA complements the moderate specificity of GDH. Key barriers to CDI testing using guideline-recommended methods were centered around awareness and resource limitations. This study from Saudi Arabia demonstrated several similarities with previous studies examining *C.*

difficile testing practices across multiple countries. The use of EIAs and NAATs resembles studies from England, Canada, and Australasia, indicating that they are widely adopted diagnostic techniques globally [10,12-14]. For example, NAATs were the most utilized test in Saudi Arabia (22.7 %) and Canada (69.8 %), while EIAs were more common in England (53 %). However, the distribution and combination of EIAs versus NAATs varied greatly between regions and periods based on various factors. These may include differences in local guidelines, cost constraints, and evolution in preferred diagnostic algorithms. While NAAT adoption is rising, some laboratories still rely heavily on EIAs due to their rapid turnaround time and low cost, especially in resource-limited settings [4]. Also, limited funding and resources were frequently cited barriers to optimal testing among many studies, including studies from Ireland and Canada [13,15]. This highlights how inadequate funding and infrastructure commonly hinder laboratories globally from implementing ideal CDI diagnostic protocols. Furthermore, this study, as well as studies from Spain, Canada and Australia, demonstrated that large hospitals tend to report more routine testing and identification of positive CDI cases compared to small hospitals [13,14]. This suggests that patient volumes and resource availability enable more extensive CDI diagnosis in bigger facilities across multiple global regions, consistent with patterns observed across several countries. This study revealed that 25.8 % of respondents were unsure which tests they used, representing a concerning practical experience gap. This uncertainty regarding testing practices was not evident in English studies, suggesting better awareness in those locations [12].

A key finding in this study was that large hospitals were significantly more likely to routinely report positive *C. difficile* cases compared to smaller hospitals ($p < 0.001$), suggesting a potential underdiagnosis in smaller facilities. Also, most large hospitals test specimens at least monthly, while most small hospitals never receive specimens. These results reveal suboptimal testing frequencies, with 38.9 % of small hospitals never receiving stool specimens and only 18.8 and 21.9 % of large hospitals receiving specimens only weekly or monthly. This suggests potential underdiagnosis of CDIs, as regular stool testing is required for timely and accurate detection. Alternatively, this may be explained by the low incidence of CDI in Saudi Arabia as reported in previous studies; though, the possibility of underdiagnosis in these studies cannot be ruled out [3,16]. High percentage of large hospitals reported never receiving stool specimens for CDI testing. Potential factors contributing to limited stool testing even in well-resourced hospitals include over-reliance on external referral, high thresholds for testing due to cost constraints, and inadequate clinician ordering practices. However, limitations of self-reported survey data, prone to misinterpretation, cannot be ruled out. Quantifying and comparing the actual stool volumes tested across hospital laboratories in future studies could provide more objective insights. Despite these concerns, the findings indicated that missed diagnoses are likely even in larger hospitals, which may underscore the need for ongoing education and stewardship to optimize CDI testing adherence.

Study from Canada found that CDI incidence correlated with hospital size, which differs from findings of this present study [13]. This discrepancy may be due to differences in healthcare systems, infrastructure, testing practices between countries, and the low incidence of CDI. Significantly, these previous studies had more than two categories of hospital bed sizes, which increases CDI incidence. Nonetheless, study from Spain raised the concern of potential underdiagnosis using EIA alone. However, the study indicated no difference in perceived barriers like limited funding based on hospital size, disagreeing with previous study conducted in Canada [13]. Overreliance on molecular tests without sufficient use of evidence-based multistep algorithms represents a missed opportunity to maximize accuracy. Low positivity rates raise underdiagnosis concerns. Therefore, significant opportunities exist to enhance CDI diagnosis in Saudi Arabia through education, evidence-based testing algorithms, addressing resource constraints, and protocol standardization.

Strengths and limitations of the study

This study provides the first national-level data on CDI testing practices across Saudi Arabian laboratories. The study collected specific, measurable data on laboratory testing volumes (with actual numbers reported), current diagnostic methods in use, existing testing protocols, and reported barriers to testing. With broad geographical coverage, the results are more generalizable across Saudi Arabia. The use of previously validated survey questions, further reviewed by infectious disease experts, also strengthened the study.

However, there were limitations. The sample size was much smaller at just 68 respondents from Saudi Arabia compared to more than 100

laboratories included in most national surveys from other countries. However, given the limited number of laboratories in Saudi Arabia, this sample size is unlikely to affect the representativeness and generalizability of the results, especially as the number of participants met the target calculated sample size based on the total number of hospitals in Saudi Arabia. Also, the study relied on self-reported data from laboratories, which may introduce reporting bias and inaccuracies. Furthermore, the study did not account for hospital characteristics including location, patient demographics, and infection control practices. While SCFHS maintains a registry of healthcare practitioners, including microbiologists, the exact number of actively practicing hospital microbiologists in Saudi Arabia at the time of the survey distribution was not available to the research team. This limitation may impact the generalizability of our findings. Despite these limitations, the findings are robust providing direction to all key stakeholder groups in Saudi Arabia. Future research may benefit from collaborative efforts with regulatory bodies to obtain more precise workforce data, enabling more accurate response rate calculations.

CONCLUSION

This survey reveals that CDI testing in microbiology laboratories in Saudi Arabia follows international guideline recommendations, albeit very few utilize the most accurate multistep methods. Poor awareness, infrequent stool testing, practical experience gaps, and resource constraints are major barriers in CDI diagnostic practices in Saudi Arabia. Improving testing practices could strengthen surveillance, improve patient outcomes, and reduce CDI burden.

Recommendations

This study recommended the development of standardized testing procedures in Saudi Arabia to ensure consistent and accurate diagnoses, promoting the use of EIA, establishing surveillance systems, forming a CDI working group to review testing methodologies and make evidence-based recommendations for best practices in diagnosing CDI, molecular characterization of CDI isolates, regular data collection and analysis to identify trends, track the effectiveness of interventions, and make necessary adjustments to practices; regular review to examine current curricula for training microbiologists and laboratory staff and regular education and training post-qualification to laboratory staff to ensure they are up to date with the latest testing methodologies and best practices.

DECLARATIONS

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Conflict of Interest

No conflict of interest is associated with this work

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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