American Journal of Biochemistry and Biotechnology

Research Article

Trends in the Prevalence of *Clostridioides difficile* Using Real-Time PCR in South Korea (2020-2024)

[1]Yonghee Lee, [2]Yoo Na Chung and [1]Jae Kyung Kim

[1]Department of Biomedical Laboratory Science, College of Health & Welfare, Dankook University, Republic of Korea
[2]Department of Laboratory Medicine, Dankook University Hospital, College of Medicine, Dankook University, Republic of Korea

Article history
Received: 11-12-2024
Revised: 25-03-2025
Accepted: 26-04-2025

Corresponding Author:
Jae Kyung Kim
Department of Biomedical Laboratory Science, College of Health & Welfare, Dankook University, Republic of Korea
Email: nerowolf2@dankook.ac.kr

Abstract: *Clostridioides difficile* is a major cause of infection in healthcare settings, and frequent antibiotic use exacerbates its occurrence. *C. difficile* infection (CDI) is a major public-health challenge owing to its complications and high mortality rate. We aimed to analyze current trends in *C. difficile* prevalence. This study was a retrospective, single center analysis of 7,371 samples from April 2020 to March 2024. Toxigenic *C. difficile* was identified from stool samples of inpatients and outpatients using an Xpert *C. difficile* assay by detecting binary toxin and toxin B via real-time polymerase chain reaction. Prevalence rates were analyzed according to sex, time of occurrence, and age using SPSS Version 29. The chi-square test and the exact test Monte Carlo method were used. Cross-analyses revealed no significant correlation between the occurrence of CDI and months, as well as individual quarters. However, when aggregating data from the same quarter across different years, significant correlations were observed ($p = 0.036$). Sex-specific prevalence analysis revealed positivity rates of 16.9% and 15.6% in males and females, respectively; however, this difference was not statistically significant. The analysis of prevalence by age did not show a statistically significant difference in cross-analysis. Nevertheless, there was a trend indicating that the positivity rate increased with advancing age. Specifically, the average age of patients with positive results was 72.6 years. This study offers baseline data on the current prevalence of CDI, serving as a valuable resource for hospitals in developing infection control plans and prevention strategies. Additionally, it provides critical insights into CDI epidemiology in South Korea, particularly the increased vulnerability to infection among older age groups. These findings emphasize the need for customized treatment and prevention strategies tailored to the older population, contributing to more effective healthcare interventions and improved patient outcomes.

Keywords: *Clostridioides difficile*, Healthcare-Associated Infection, Prevalence, Real-time PCR, Seasonal Analysis

Introduction

Clostridioides difficile is a major cause of healthcare-associated infections worldwide and poses significant risks, including pseudomembranous colitis and sepsis, particularly in older adults and immunocompromised individuals (Marra *et al.*, 2020; Alharbi *et al.*, 2021; Feuerstadt *et al.*, 2021). The incidence of *C. difficile* infection (CDI) is increasing, creating major public-health challenges (Lessa *et al.*, 2015; Rao and Malani, 2020) regardless of close monitoring in hospitals (Fu *et al.*, 2021; Dilnessa *et al.*, 2022). Maintaining cleanlines and adhering to management guidelines help prevent infection (Fonseca *et al.*, 2024). Understanding CDI epidemiology through continuous monitoring is crucial for designing such guidelines. While CDI has been extensively studied in Western countries, relatively fewer studies have explored its epidemiology in Asian populations. South Korea, with its high antibiotic prescription rates and aging population, presents a unique setting for CDI research. Furthermore, infection control measures in South Korea, such as national surveillance programs, may offer insights that are applicable to other countries. By analyzing recent CDI

trends in this study, we aimed to contribute to a broader understanding of global CDI epidemiology and infection control policies.

Materials and Methods

Patients and Data Collection

Between April 2020 and March 2024, 7,371 stool samples from inpatients, outpatients, and emergency room patients with complaints of abdominal pain or diarrhea, as well as immunocompromised patients, at Kyung Hee University Hospital, were analyzed using automated real-time Polymerase Chain Reaction (PCR)—a method that allows rapid and accurate detection of CDI (Jensen *et al.*, 2015; Kim *et al.*, 2017). Due to a shortage of test kits at our facility, from January to March 2022, data were outsourced. The outsourced test data were excluded due to differences in laboratory protocols, including variations in sample processing times and reagents. Including this data could have introduced inconsistencies, potentially affecting the accuracy of prevalence estimates. Of the 7,371 analyzed samples, 1,196 were positive for CDI. The data of these positive samples were retrospectively examined for prevalence patterns according to sex, age, and season, and the results were compared with those of previous studies. Duplicate patient data were excluded.

Detection of C. difficile Toxin B, Binary Toxin, and tcdC Nucleotide Deletion 117

Stool samples were collected using sterile spoons and stored in sterile containers. All samples were immediately stored at 4°C and tested within 2 h. An Xpert *C. difficile* assay kit (Cepheid, Sunnyvale, CA, USA), with the GeneXpert instrument (Cepheid) and GeneXpert Dx System (Cepheid), was used to detect *C. difficile* toxin B and binary toxin. All reagents were stored at room temperature (20–30°C) and used within their effective period.

Analysis Method

A swab was briefly immersed in the stool sample and inserted into a sample reagent tube. The swab stem was broken off, and the tube was tightly closed and vortexed at high speed. The cartridge lid was opened, and the sample was transferred to the "S" chamber of the Xpert *C. difficile* cartridge using a pipette tip. Then, the cartridge lid was closed. In the GeneXpert System, a test was created. "Start Test" was clicked, the cartridge was loaded, and the door was closed to begin the test.

Validity

The Cycle threshold (Ct) value of the control sample was considered valid when it was within the range specified by the manufacturer (a total of 40 cycles, toxin B [5–37], binary toxin [5–37], *tcdC* deletion nucleotide 117 [5–40], Sample Processing Control [SPC; 5–40]). All tests included an SPC and Probe Check, and the amplification curves were reviewed.

Interpretation

The results were interpreted based on fluorescent signals and calculation algorithms of the GeneXpert Instrument System, summarized in the "View Results" window as follows:

Toxigenic *C. difficile* POSITIVE, 027 PRESUMPTIVE NEG: Toxin B or toxin B plus either binary toxin (or *tcdC* deletion nucleotide 117) was detected with valid Ct values. SPC was ignored if *C. difficile* amplification competed with it. Probe Check passed.

Toxigenic *C. difficile* POSITIVE, 027 PRESUMPTIVE POS: Toxin B and binary toxin (or *tcdC* deletion nucleotide 117) were detected with valid Ct values. SPC was ignored if *C. difficile* amplification competed with it. Probe Check passed.

Toxigenic *C. difficile* NEGATIVE, 027 PRESUMPTIVE NEG: No toxin B or other *C. difficile* target DNA was detected. SPC met the acceptance criteria. Probe Check passed.

Statistical Analysis

Data were analyzed using SPSS software (version 29.0; IBM, Armonk, NY, USA). Data are reported as frequencies (percentages). We analyzed the correlation between the prevalence of CDI and sex, age, incidence, and time. We performed a cross-analysis by month and quarters and categorized the age groups into 10-year intervals ranging from <10 years to >70 years. Fisher's exact test and the chi-square test, with the Monte Carlo method to ensure accuracy for cells with expected values <5, were performed to assess CDI prevalence patterns by season, sex, and age. Statistical significance was set at a p-value <0.05.

Although primary trend analyses were conducted using quarterly data for statistical stability, monthly variations in CDI prevalence were also analyzed and are presented in the Results section. While a separate table for monthly trends is not included, these fluctuations are discussed in the interpretation of the results.

Sensitivity Analysis

In this study, we performed a sensitivity analysis to evaluate the impact of missing data for the period from January to March 2022 on the study's conclusions. The missing data was imputed using the average values from January to March in the years 2021, 2023, and 2024. Specifically, we used the mean values for the variables of interest (e.g., CDI positivity rate) from these periods to replace the missing data. The imputed dataset was then compared with the original dataset that contained the missing data to assess the effect on the study's results.

Results

Cross-Analysis According to Months

The cross-analysis of CDI prevalence according to months analyzed CDI positivity data by year for each month. The chi-square value was 52.704 (p = 0.173), indicating no significant correlation between CDI prevalence and individual months. Similarly, aggregating data for the same month across years yielded a non-significant chi-square value of 15.474 (p = 0.162).

Cross-Analysis According to Quarters

Analyzing CDI positivity rates using data for individual quarters across multiple years resulted in a Pearson's chi-square value of 21.220 (p = 0.130), indicating no significant correlation. However, when analyzing the aggregated data for the same quarters over several years, a significant correlation was observed (chi-square = 8.545, p = 0.036).

Table 1: Quarterly *Clostridioides difficile* infection prevalence data (2020–2024)

Date (quarter)	Total number of cases	Positive cases	Positivity rate (%)
Q2 2020	328	49	14.9
Q3 2020	393	73	18.6
Q4 2020	365	69	18.9
Q1 2021	413	63	15.3
Q2 2021	523	76	14.5
Q3 2021	266	37	13.9
Q4 2021	512	77	15.0
Q1 2022	10	1	10.0
Q2 2022	440	61	13.9
Q3 2022	574	91	15.9
Q4 2022	590	101	17.1
Q1 2023	526	87	16.5
Q2 2023	612	85	13.9
Q3 2023	635	129	20.3
Q4 2023	669	102	15.2
Q1 2024	515	92	17.9
Total	7371	1193	16.2

Prevalence of CDI by Season of Incidence

Of the 7,371 samples screened, 1,193 tests were positive for toxin B (the binary toxin was detected simultaneously with toxin B). The highest positivity rate was observed in July 2023, with a rate of 22.2% (39 positive cases), followed by April 2020 at 22.1% (19 positive cases) and December 2020 at 21.5% (26 positive cases). Seasonally, the third quarter of 2023 had the highest positivity rate at 20.3% (129 cases), followed by the fourth quarter of 2020 at 18.9% (69 cases) and the third quarter of 2020 at 18.6% (73 cases) (Table 1).

Prevalence by Sex

Out of the 7,371 samples screened, 3,577 (48.5%) were from males and 3,794 (51.5%) from females. CDI positivity was observed in 603 (16.9%) males and 590 (15.6%) females. Statistical analysis using the chi-square test yielded a value of 2.318 (p = 0.128), indicating no significant association between sex and CDI positivity (Figure 1).

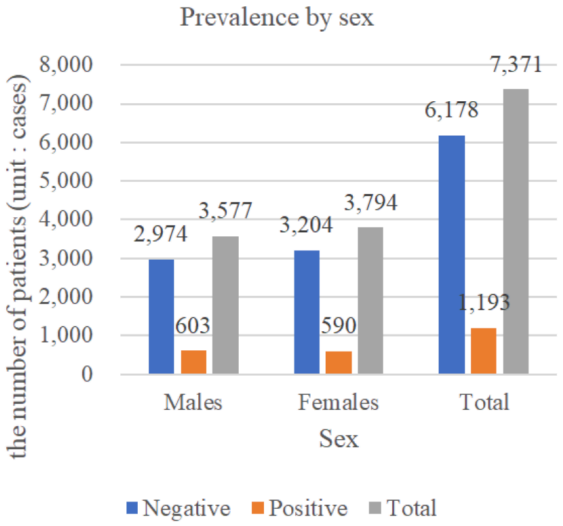

Fig. 1: Graph of *Clostridioides difficile* infection results by sex

Prevalence by Age

Cross-analysis was conducted by grouping patients by age to examine the association between age and CDI positivity. Pearson's chi-square value was 7.494 (p = 0.379), and the Monte Carlo exact test showed a significance probability of 0.394, with a 99.9% confidence interval of 0.394–0.395 based on 10,000,000 simulations, indicating that age and CDI positivity were not significantly related.

The highest positivity rate was observed in individuals in their teens (23.5%, four cases), followed by those aged >70 years (16.6%, 796 cases) and those in their 60s (16.3%, 206 cases). The mean age of patients with CDI was 72.6±14.8 years (Table 2).

Table 2: Prevalence of *Clostridioides difficile* infection by age group (2020–2024)

Age range (years)	Total number of cases	Positive case	Positive rate (%)
<10	3	0	0.0
10–19	17	4	23.5
20–29	169	21	12.4
30–39	184	22	12.0
40–49	318	44	13.8
50–59	629	100	15.9
60–69	1,266	206	16.3
>70	4,785	796	16.6
Total	7,371	1,196	16.2

Sensitivity Analysis

The sensitivity analysis revealed that imputing the missing data with the average values from 2021, 2023, and 2024 did not result in any significant differences in

the study's findings. Specifically, we conducted Chi-Square analysis to compare the distribution of CDI positivity rates between the imputed and original datasets. The Chi-Square tests were performed for the following categorical variables: Sex and CDI positivity rate, where the p-value changed from 0.128 in the original dataset to 0.152 in the imputed dataset, showing no significant differences; Age group and CDI positivity rate, with the p-value changing from 0.379 in the original dataset to 0.391 in the imputed dataset, indicating no significant differences; and Prevalence of CDI over Seasons, where monthly analysis showed a change in the p-value from 0.173 to 0.157. When the months were combined for seasonal analysis, the p-value shifted from 0.162 to 0.155, and in quarterly analysis, the p-value changed from 0.130 to 0.117 for each individual quarter. When the quarters were aggregated, the p-value changed from 0.036 to 0.032, indicating that the sensitivity analysis did not significantly affect the conclusions of the study.

Discussion

Prevalence of CDI Over Seasons

Our study revealed significant seasonal variation in CDI prevalence. The assessment of the impact of seasonal factors on CDI occurrence revealed that the highest positivity rate was observed in July 2023 (22.2%, 39 cases), followed by April 2020 (22.1%, 19 cases) and December 2020 (21.5%, 26 cases).

An analysis of aggregated monthly data revealed that July had the highest positivity rate for CDI (18.9%, 138 cases), followed by January (18.5%, 99 cases) and March (17.3%, 73 cases). Analysis according to months was influenced by the volume of testing, making it difficult to attribute changes in prevalence to specific months. Cross-analysis indicated that the monthly variations in CDI prevalence were not statistically significant.

Analysis according to quarters revealed the highest positivity rate in the third quarter of 2023 (20.3%, 129 cases), followed by the fourth quarter of 2020 (18.9%, 69 cases) and the third quarter of 2020 (18.6%, 73 cases). However, owing to the variability in the number of positive cases, these results might lack statistical robustness due to sample size variability and methodological limitations. Moreover, cross-analysis showed no significant relationship between quarters and CDI incidence. Nevertheless, aggregated data of quarters across different years revealed a significant association of quarters with CDI prevalence, indicating the influence of seasonal variation.

These results differ from those of previous studies, which reported no relationship between CDI occurrence and seasonality (de Jager *et al.*, 2021; Maestri *et al.*, 2022). However, several other studies have reported that seasonality affects occurrence (Argamany *et al.*, 2015; Lee *et al.*, 2016; Piatti *et al.*, 2017). While our findings on seasonal trends align with those of previous studies, our study uniquely provides detailed analyses of age-specific CDI prevalence and sex-based differences. Furthermore, by emphasizing the role of real-time PCR in CDI detection, our study reinforces the importance of molecular diagnostic techniques in modern epidemiology. Our results highlight seasonal variations in CDI prevalence. However, additional environmental factors may also influence infection rates. Prior studies link hospital-based conditions—such as temperature and humidity—to *C. difficile* carriage (Muñoz-Price *et al.*, 2020), while community-level hygiene may affect CDI incidence outside hospital settings (Bloomfield and Riley, 2016). Future research should incorporate metrics like humidity logs or bed occupancy to provide a more holistic view of CDI epidemiology. Differences in various environmental factors (Guh *et al.*, 2017), antibiotic use, underlying diseases, and hospital environments between studies complicate the assessment of changes in CDI prevalence. In addition, the limited number of studies on the seasonality of CDI in South Korea poses challenges for comparison with previous research. Therefore, we intend to contribute to the development of a plan for continuous monitoring of CDI prevalence.

Although significant differences were found when aggregating data for the same quarters across different years—as can be seen in Table 1—even in the same quarter, there were substantial differences across the years. Therefore, we believe that this approach cannot be applied unconditionally. Moreover, as statistical and clinical significance do not always correspond, it would be ideal to investigate CDI prevalence by conducting further research in several institutions over a longer period, rather than relying exclusively on the findings of a single-institution study conducted over a short time.Recent studies from Europe and North America suggest differing seasonality patterns for CDI. While some studies report increased incidence in winter, others find no seasonal effect. Our findings align more closely with those of Asian studies, highlighting possible regional variations. For instance, while studies from the United States and Canada report increased CDI incidence during winter, research from certain Asian countries, including Japan, suggests no clear seasonal trend. Our findings align more closely with those of Asian studies, indicating potential regional differences in CDI epidemiology. While this study presents primary findings in quarterly intervals, to enhance statistical reliability, monthly data analysis was conducted to account for finer variations and is discussed in the Results section. Future studies should consider incorporating separate tabular presentations of monthly trends for additional clarity.

Prevalence by Sex

The present study identified minimal differences in the number of test requests and positivity rates between

males and females, and the analyses revealed no significant sex-based associations. Most previous studies (Lin *et al.*, 2015; Mawer *et al.*, 2017; Lee *et al.*, 2019; Le Monnier *et al.*, 2022; Moon *et al.*, 2022) have also reported no significant correlation between sex and CDI prevalence. However, Reigadas *et al.* (2017) reported a significant correlation, suggesting that further research is required to integrate the findings from various regions and institutions.

Figure 1 shows the CDI positivity rate according to sex. No significant difference was observed, but a slightly higher prevalence was observed in men, indicating that sex may have a potential impact on prevalence; however, this finding differs from that of previous studies suggesting potential biological or medical access differences (Esteban-Vasallo *et al.*, 2016).

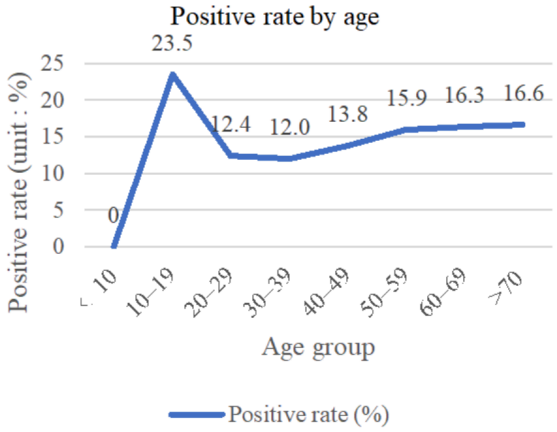

Fig. 2: *Clostridioides difficile* infection positivity rate by age

Prevalence by Age

Regarding age, the highest positivity rate was observed in the teenage group (23.5%). Regarding the teenage group's unexpectedly high positivity rate, it is crucial to note that a small sample size (n=17) may inflate percentages. Additionally, adolescent patients may present different clinical characteristics or seek medical care under distinct criteria. To confirm whether this observation represents a meaningful trend or a statistical anomaly, future studies should recruit larger sample sizes across multiple institutions and collect more detailed clinical data on younger patients (e.g., antibiotic history, immunization status, or underlying conditions). The average age of patients with CDI positivity was 72.6 years. The observed high CDI prevalence in older adults may be influenced by increased diagnostic testing rather than a true increase in infection rates. Future studies should adjust for testing frequency to confirm these findings. Previous studies have reported that CDI incidence was independent of age (Wenisch *et al.*, 2012; Salazar *et al.*, 2017; Muñoz-Price *et al.*, 2020; Le Monnier *et al.*, 2022). However, some studies (Starr *et al.*, 2003; Song and Kim, 2019) have suggested that older adults are at higher risk for infection and have poorer outcomes (Reigadas *et al.*, 2017). Thus, continued epidemiological research and preventive measures are recommended for older adults (Figure 2).

Strengths and Limitations of the Study

This study offers valuable insights into the prevalence of CDI across different periods, sexes, and age groups. However, it has some limitations. First, this study used data from a single medical institution, which may limit its generalizability. Future multicenter studies are necessary to confirm whether these trends persist across different hospitals and regions. Second, this study excluded data from January to March 2022 due to methodological inconsistencies arising from outsourced testing. A comparison with adjacent months showed no major discrepancies, supporting the reliability of our trend analysis. While this creates a temporary gap in the data collection, the overall prevalence trend remains unaffected, as confirmed by the consistency of results in subsequent quarters. Third, the seasonal analysis is limited by sample size variability, which may affect statistical significance. Future studies should incorporate larger datasets to validate these trends and improve statistical power. Fourth, Due to the absence of detailed clinical data (e.g., antibiotic history, comorbidities, admission type), we could not perform a multivariate analysis to control for confounding factors. Future research should collect such information to allow logistic regression or other advanced modeling techniques, thereby strengthening the conclusions on CDI risk factors. Although seasonal trends in CDI prevalence were analyzed, this study did not incorporate environmental factors such as humidity, temperature, or hospital-related variables such as overcrowding and bed occupancy rates. These factors may influence CDI transmission and prevalence. Future studies should integrate these elements to provide a more comprehensive assessment of CDI seasonality and potential outbreak triggers. Additionally, we focused solely on toxin B and binary toxin detection due to their primary role in CDI pathogenesis; this might not represent the full spectrum of CDI prevalence. Future studies should incorporate multivariate analysis to account for these confounding variables and include toxin A detection and strain typing for a more comprehensive epidemiological analysis. Fifth, this study did not include longitudinal follow-up to assess CDI recurrence or long-term outcomes. Future research should incorporate patient tracking to better understand disease progression and treatment effectiveness. Furthermore, although the four-year period provides valuable epidemiological insights, long-term surveillance is necessary to confirm sustained CDI trends. Sixth, we employed the chi-square test and Monte Carlo method for categorical data analysis, as they are well-suited for identifying prevalence differences in grouped data. Given the sample size variations across categories, these methods provided reliable estimates. While some

associations were statistically significant, their clinical relevance should be assessed in future studies to determine their impact on patient care and treatment outcomes. However, we acknowledge that logistic regression could better control for confounding factors, and we recommend its application in future studies to further validate our findings.

Despite its limitations, this study makes a valuable contribution to infection control and prevention strategies by identifying seasonal patterns and highlighting the higher occurrence of positive cases among older adults. It provides important baseline data on CDI prevalence in South Korea and offers directions for future research. To overcome the current limitations and achieve a deeper understanding of CDI prevalence, follow-up studies, particularly multicenter investigations and multivariate analyses, are warranted. Such efforts could enhance patient management and strengthen healthcare system efficiency in the face of the growing risk of CDI (Son *et al.*, 2020).

Sensitivity Analysis

The results of the sensitivity analysis suggest that the missing data for the period from January to March 2022 did not have a significant impact on the conclusions of this study. The imputed values, based on the average data from 2021, 2023, and 2024, did not lead to substantial changes in the statistical outcomes. This indicates that the study's conclusions are robust, even in the presence of missing data, and the imputation method used was appropriate. However, it is recommended that future studies with more complete data should be conducted to further validate these findings.

Interpretation and Implications

The findings of this study may have practical implications for patient management, particularly for older adults for whom higher positivity rates were observed. A tailored approach based on age may enhance infection prevention and control strategies. Additionally, recognizing seasonal prevalence patterns could lead to more vigilant monitoring of patients during periods of increased infection risk. Such clinical insights may guide future patient care strategies.

Conclusion

This study highlights *Clostridioides difficile* prevalence trends in South Korea using real-time PCR, providing rapid and accurate diagnostics. By analyzing four years of data, we identified seasonal trends and an age-specific prevalence, emphasizing the need for continuous CDI surveillance. However, the exclusion of outsourced test data (January–March 2022) may have introduced minor inconsistencies, and confounding factors such as antibiotic use and comorbidities were not assessed. Expanding this study to multiple institutions will enhance generalizability, and integration of clinical data such as antibiotic stewardship programs and hospital environmental factors, analyzed using multivariate analysis, could provide deeper insights into CDI transmission dynamics. Long-term surveillance and predictive modeling may further refine infection control strategies, ultimately reducing CDI burden and improving patient outcomes.

Acknowledgment

We thank Dr Young Jin Kim for their assistance in data entry.

Funding Information

This study did not receive any funding.

Conflict of Interest

The authors have no conflicts of interests to declare.

Author's Contributions

Yonghee Lee: Performed data collection and interpretation, contributed to manuscript writing, and approved the final version.

Yoo Na Chung: Contributed to study design, participated in manuscript writing, data interpretation, and final approval.

Jae Kyung Kim: Conceived and designed the study, contributed to data interpretation, and critically revised the manuscript.

All authors participated in the final interpretation of the data, provided individual expertise, and reviewed the manuscript for important intellectual content.

Equal Contribution: Yoo Na Chung and Yonghee Lee contributed equally to this work and share first co-authorship.

Ethics

This study was approved by the Dankook University Institutional Review Board (approval IRB no.: DKU 2024-10-007-002) and adhered to the principles embodied in the Declaration of Helsinki. Since this study involved a retrospective analysis of pre-existing anonymous test results, the requirement for informed consent was waived.

References

Alharbi, A. K., Ahmed, M. A., Tashkandi, A., Alkhathaami, F. A., & Alshehri, A. I. (2021). Persistent Clostridium Difficile Diarrhea, Thinking Beyond Pseudomembranous Colitis: A Case Report. *Cureus*, *13*(12), e20704. https://doi.org/10.7759/cureus.20704

Argamany, J. R., Aitken, S. L., Lee, G. C., Boyd, N. K., & Reveles, K. R. (2015). Regional and Seasonal Variation in Clostridium Difficile Infections Among Hospitalized Patients in the United States, 2001-2010. *American Journal of Infection Control*, *43*(5), 435–440. https://doi.org/10.1016/j.ajic.2014.11.018

Bloomfield, L. E., & Riley, T. V. (2016). Epidemiology and Risk Factors for Community-Associated Clostridium Difficile Infection: A Narrative Review. *Infectious Diseases and Therapy*, *5*(3), 231–251. https://doi.org/10.1007/s40121-016-0117-y

de Jager, P., Smith, O., Bolon, S., Thomas, J., & Richards, G. A. (2021). Epidemiology of *Clostridioides difficile* in South Africa. *PLOS ONE*, *16*(11), e0259771. https://doi.org/10.1371/journal.pone.0259771

Dilnessa, T., Getaneh, A., Hailu, W., Moges, F., & Gelaw, B. (2022). Prevalence and Antimicrobial Resistance Pattern of Clostridium Difficile Among Hospitalized Diarrheal Patients: A Systematic Review and Meta-Analysis. *PLOS ONE*, *17*(1), e0262597. https://doi.org/10.1371/journal.pone.0262597

Esteban-Vasallo, M. D., Naval Pellicer, S., Domínguez-Berjón, M. F., Cantero Caballero, M., Asensio, Á., Saravia, G., & Astray-Mochales, J. (2016). Age and Gender Differences in Clostridium Difficile-Related Hospitalization Trends in Madrid (Spain) over a 12-Year Period. *European Journal of Clinical Microbiology and Infectious Diseases*, *35*(6), 1037–1044. https://doi.org/10.1007/s10096-016-2635-7

Feuerstadt, P., Boules, M., Stong, L., Dahdal, D. N., Sacks, N. C., Lang, K., & Nelson, W. W. (2021). Clinical Complications in Patients with Primary and Recurrent *Clostridioides difficile* Infection: A Real-World Data Analysis. *SAGE Open Medicine*, *9*. https://doi.org/10.1177/2050312120986733

Fonseca, F., Forrester, M., Advinha, A. M., Coutinho, A., Landeira, N., & Pereira, M. (2024). *Clostridioides difficile* Infection in Hospitalized Patients—A Retrospective Epidemiological Study. *Healthcare*, *12*(1), 76. https://doi.org/10.3390/healthcare12010076

Fu, Y., Luo, Y., & Grinspan, A. M. (2021). Epidemiology of Community-Acquired and Recurrent *Clostridioides difficile* Infection. *Therapeutic Advances in Gastroenterology*, *14*. https://doi.org/10.1177/17562848211016248

Guh, A. Y., Adkins, S. H., Li, Q., Bulens, S. N., Farley, M. M., Smith, Z., Holzbauer, S. M., Whitten, T., Phipps, E. C., Hancock, E. B., Dumyati, G., Concannon, C., Kainer, M. A., Rue, B., Lyons, C., Olson, D. M., Wilson, L., Perlmutter, R., Winston, L. G., … McDonald, L. C. (2017). Risk Factors for Community-Associated Clostridium Difficile Infection in Adults: A Case-Control Study. *Open Forum Infectious Diseases*, *4*(4), ofx171. https://doi.org/10.1093/ofid/ofx171

Jensen, M. B. F., Olsen, K. E. P., Nielsen, X. C., Hoegh, A. M., Dessau, R. B., Atlung, T., & Engberg, J. (2015). Diagnosis of Clostridium Difficile: Real-Time PCR Detection of Toxin Genes in Faecal Samples is More Sensitive Compared to Toxigenic Culture. *European Journal of Clinical Microbiology and Infectious Diseases*, *34*(4), 727–736. https://doi.org/10.1007/s10096-014-2284-7

Kim, K.-B., Kim, D. H., Lee, W., Ha, J.-S., Ryoo, N.-H., Jeon, D.-S., & Kim, J.-R. (2017). Comparison and Evaluation of Diagnostic Assays for Clostridium Difficile Infection. *Laboratory Medicine Online*, *7*(2), 73–78. https://doi.org/10.3343/lmo.2017.7.2.73

Le Monnier, A., Candela, T., Mizrahi, A., Bille, E., Bourgeois-Nicolaos, N., Cattoir, V., Farfour, E., Grall, I., Lecointe, D., Limelette, A., Marcade, G., Poilane, I., Poupy, P., Kansau, I., Zahar, J. R., Pilmis, B., & Group, G. M. C. (2022). One-day prevalence of asymptomatic carriage of toxigenic and non-toxigenic *Clostridioides difficile* in 10 french hospitals. *Journal of Hospital Infection*, *129*, 65–74. https://doi.org/10.1016/j.jhin.2022.05.011

Lee, H.-Y., Hsiao, H.-L., Chia, C.-Y., Cheng, C.-W., Tsai, T.-C., Deng, S.-T., Chen, C.-L., & Chiu, C.-H. (2019). Risk Factors and Outcomes of Clostridium Difficile Infection in Hospitalized Patients. *Biomedical Journal*, *42*(2), 99–106. https://doi.org/10.1016/j.bj.2018.12.002

Lee, J.-C., Hung, Y.-P., Lin, H.-J., Tsai, P.-J., & Ko, W.-C. (2016). Clostridium Difficile Infections in Medical Intensive Care Units of a Medical Center in Southern Taiwan: Variable Seasonality and Disease Severity. *PLOS ONE*, *11*(8), e0160760. https://doi.org/10.1371/journal.pone.0160760

Lessa, F. C., Mu, Y., Bamberg, W. M., Beldavs, Z. G., Dumyati, G. K., Dunn, J. R., Farley, M. M., Holzbauer, S. M., Meek, J. I., Phipps, E. C., Wilson, L. E., Winston, L. G., Cohen, J. A., Limbago, B. M., Fridkin, S. K., Gerding, D. N., & McDonald, L. C. (2015). Burden of Clostridium Difficile Infection in the United States. *New England Journal of Medicine*, *372*(9), 825–834. https://doi.org/10.1056/nejmoa1408913

Lin, H.-J., Hung, Y.-P., Liu, H.-C., Lee, J.-C., Lee, C.-I., Wu, Y.-H., Tsai, P.-J., & Ko, W.-C. (2015). Risk Factors for Clostridium Difficile-Associated Diarrhea Among Hospitalized Adults with Fecal Toxigenic C. Difficile Colonization. *Journal of Microbiology, Immunology and Infection*, *48*(2), 183–189. https://doi.org/10.1016/j.jmii.2013.08.003

Maestri, A. C., Ando, P. K., Sarot, G., de Castilho, F., Raboni, S. M., & Nogueira, K. S. (2022). Prevalence and Seasonality of *Clostridioides difficile* over 12 Years at a Tertiary Hospital in Brazil. *Current Microbiology*, *79*(12), 354. https://doi.org/10.1007/s00284-022-03062-6

Marra, A. R., Perencevich, E. N., Nelson, R. E., Samore, M., Khader, K., Chiang, H.-Y., Chorazy, M. L., Herwaldt, L. A., Diekema, D. J., Kuxhausen, M. F., Blevins, A., Ward, M. A., McDanel, J. S., Nair, R., Balkenende, E., & Schweizer, M. L. (2020). Incidence and Outcomes Associated With Clostridium Difficile Infections A Systematic Review and Meta-Analysis. *JAMA Network Open*, *3*(1), e1917597. https://doi.org/10.1001/jamanetworkopen.2019.17597

Mawer, D. P. C., Eyre, D. W., Griffiths, D., Fawley, W. N., Martin, J. S. H., Quan, T. P., Peto, T. E. A., Crook, D. W., Walker, A. S., & Wilcox, M. H. (2017). Contribution to Clostridium Difficile Transmission of Symptomatic Patients With Toxigenic Strains Who Are Fecal Toxin Negative. *Clinical Infectious Diseases*, *64*(9), 1163–1170. https://doi.org/10.1093/cid/cix079

Moon, S. H., Kim, H. H., Nam, D.-H., Moon, Y., Kang, M.-G., Choi, I. Y., Byeon, S., Yang, H.-J., Kim, S.-H., Kim, K., & Kang, D. Y. (2022). Risk Factors for Clostridium Difficile Infection in Antibiotic Use Inpatients Based on Common Data Model. *Journal of Pharmacoepidemiology and Risk Management*, *14*(1), 86–92. https://doi.org/10.56142/2022.14.1.86

Muñoz-Price, L. S., Hanson, R., Singh, S., Nattinger, A. B., Penlesky, A., Buchan, B. W., Ledeboer, N. A., Beyer, K., Namin, S., Zhou, Y., & Pezzin, L. E. (2020). Association Between Environmental Factors and Toxigenic *Clostridioides difficile* Carriage at Hospital Admission. *JAMA Network Open*, *3*(1), e1919132. https://doi.org/10.1001/jamanetworkopen.2019.19132

Piatti, G., Bruzzone, M., Fontana, V., Mannini, A., & Ceppi, M. (2017). Epidemiology of Clostridium Difficile Infection in a Large Hospital in Northern Italy: Questioning the Ward-Based Transmission. *The Open Microbiology Journal*, *11*(1), 360–371. https://doi.org/10.2174/1874285801711010360

Rao, K., & Malani, P. N. (2020). Diagnosis and Treatment of Clostridioides (Clostridium) Difficile Infection in Adults in 2020. *JAMA*, *323*(14), 1403–1404. https://doi.org/10.1001/jama.2019.3849

Reigadas, E., AlcalÁ, L., Marín, M., Martín, A., & Bouza, E. (2017). Clinical, Immunological and Microbiological Predictors of Poor Outcome in Clostridium Difficile Infection. *Diagnostic Microbiology and Infectious Disease*, *88*(4), 330–334. https://doi.org/10.1016/j.diagmicrobio.2017.05.005

Salazar, C. L., Reyes, C., Atehortua, S., Sierra, P., Correa, M. M., Paredes-Sabja, D., Best, E., Fawley, W. N., Wilcox, M., & GonzÁlez, Á. (2017). Molecular, Microbiological and Clinical Characterization of Clostridium Difficile Isolates From Tertiary Care Hospitals in Colombia. *PLOS ONE*, *12*(9), e0184689. https://doi.org/10.1371/journal.pone.0184689

Son, K. J., Kim, Y. A., & Park, Y. S. (2020). The Trend of *Clostridioides difficile* Infection in Korean Hospitals with the Analysis of Nationwide Sample Cohort. *Annals of Clinical Microbiology*, *23*(4), 181–189. https://doi.org/10.5145/acm.2020.23.4.3

Song, J. H., & Kim, Y. S. (2019). Recurrent Clostridium Difficile Infection: Risk Factors, Treatment, and Prevention. *Gut and Liver*, *13*(1), 16–24. https://doi.org/10.5009/gnl18071

Starr, J. M., Martin, H., McCoubrey, J., Gibson, G., & Poxton, I. R. (2003). Risk Factors for Clostridium Difficile Colonisation and Toxin Production. *Age and Ageing*, *32*(6), 657–660. https://doi.org/10.1093/ageing/afg112

Wenisch, J. M., Schmid, D., Tucek, G., Kuo, H.-W., Allerberger, F., Michl, V., Tesik, P., Laferl, H., & Wenisch, C. (2012). A Prospective Cohort Study on Hospital Mortality Due to Clostridium Difficile Infection. *Infection*, *40*(5), 479–484. https://doi.org/10.1007/s15010-012-0258-1