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Epidemiology and risk factors related to Clostridiodes difficile infection in Costa Rica

Cristina Fernández Barrantes

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EPIDEMIOLOGY AND RISK FACTORS RELATED TO CLOSTRIDIODES DIFFICILE INFECTION IN COSTA RICA

Doctoral Thesis

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CERTIFY: That the present doctoral thesis, submitted by Cristina Fernández Barrantes, with the title: "Epidemiology and risk factors related to *Clostridiodes difficile* infection in Costa Rica" has been conducted under their supervision.

For the appropriate legal purposes, they sing this certificate in Barcelona, on June 4th, 2025.

Santiago Grau Cerrato Luis Esteban Hernández Soto Magí Farré Albaladejo

Department of Pharmacology, Therapeutics and Toxicology

Report presented by Cristina Fernández Barrantes for the doctoral degree at the Autonomus Universty of Barcelona.

The work" Empidemiology and risk factors related to *Clostridiodes diffcile* in Costa Rica" has been conducted at the Hospital San Juan de Dios and Hospital México of the Caja Costarricense de Seguro Social under the supervision of Dr. Santiago Grau Cerrato, Dr. Luis Esteban Hernández Soto and Dr. Magí Farré Albaladejo.

For the appropriate legal purposes, this certificate is signed in Barcelona, on June 4th, 2025.

Doctoral Candidate

Cristina Fernández Barrantes

To my parents, who taught me to trust in effort and never stop learning. And to my mentors, for their generous guidance, encouragement, and inspiration

"Great discoveries and improvements invariably involve the cooperation of many minds"—Alexander Graham Bell

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Abbreviations

AAPC: Average annual percent change

ADM: Admissions

AMC: Antimicrobial consumption

ASP: Antimicrobial stewardship program

ATC: Anatomical, therapeutical, chemical classification

ATLAS Score: Age, treatment with systemic antibiotics, leukocyte count, albumin and serum (creatinine)

AWaRe: Access, Watch, Reserve

BD: bed days

CCNA: Cytotoxicity neutralization assay

CCSS: Costa Rican Social Security Fund (Caja Costarricense de Seguro Social)

CDC: Center for Diseases Control and Prevention

CDD: Clostridiodes difficile disease

CDI: *Clostridiodes difficile* infection

CDSS: *Clostridiodes difficile* severity score

CA-CDI: Community-associated *Clostridiodes difficile* infection

CDT: *Clostridiodes difficile* transferase or binary toxin

CMV: Cytomegalovirus

DDD: Defined daily dose

DIS: Discharges

DOT: Days of therapy

EBAIS: Basic Comprehensive Health Care Teams (Equipos Básicos de Atención Integral en Salud)

ESCMID: European Society of Clinical Microbiology and Infectious Diseases

FDA: Food and Drug Administration

FMT: Fecal microbiota transplantation

GDH: Glutamate dehydrogenase

GTD: glucosyltransferase binding domin

HA-CDI: Hospital acquired *Clostridiodes difficile* infection

ICU: Intensive care unit

IDSA: Infectious Diseases Society of America

LCT: Large clostridial toxin

LOM: Official List of Medications (Lista Oficial de Mediciamentos)

LOS: Length of stay

LOT: Length of therapy

MIC: Minimum inhibitory concentration

NAAT: Acid amplification tests

NAP: North American pulsed-field gel electrophoresis type

OBD: occupied bed days

PCR: Polymerase chain reaction

PD: Patient day

PDD: Prescribed daily dose

PMC: Pseudomembranus colitis

PPI: Proton Pump Inhibitor

PSM: Propensity Score Matching

rCDI: Recurrent *Clostridiodes difficile* infection

TC: toxigenic culture

TcdA: Toxin A

TcdB: Toxin B

REA: Restriction endonuclease analysis

RNA: Ribonucleic acid

RT: ribotypes

SIFA: Integrated Pharmacy System (Sistema Integrado de Farmacias)

SHEA: Society for Healthcare Epidemiology of America

WBC: White blood cell

WHO: World Health Organization

Abstract

This doctoral research represents the first multicenter study in Latin America analyzing antimicrobial and proton pump inhibitor consumption trends and their association with hospital acquired *Clostridiodes difficile* infection (CDI) in tertiary care hospitals of the Costa Rican Social Security System. The study aimed to close a critical evidence gap by evaluating the incidence, risk factors, and clinical characteristics of hospital acquired CDI, as well as antibiotic use patterns from 2017 to 2021.

Methods

The research consisted of three components: (1) a retrospective trend analysis of antimicrobial consumption using Defined Daily Doses (DDD) per 100 bed-days and per 100 discharges from 2017 to 2021; (2) an ecological study correlating antimicrobial and proton pump inhibitors consumption with CDI incidence through time-series and Spearman correlation; and (3) a case-control study using propensity score matching to identify risk factors for nosocomial CDI in hospitalized adults from 2019 to 2021. Statistical analyses included logistic regression, Kaplan-Meier survival analysis, and segmented Poisson regression for interrupted time series.

Results

- Between 2017 and 2021, cefotaxime, vancomycin, oxacillin, and fluconazole were among the most consumed antimicrobials. Despite a decline in cephalosporin and macrolide use, consumption of carbapenems, fluoroquinolones, and vancomycin increased.
- The study identified 354 HA-CDI cases and 1172 controls. Antibiotic exposure, particularly to third-generation cephalosporins and clindamycin, was the most significant risk factor. ICU admission, prolonged hospitalization, and multiple comorbidities were also independently associated with increased HA-CDI risk.
- No statically significant association was found between proton pump inhibitors use and CDI incidence. During the COVID-19 pandemic, although antimicrobial use increased, CDI incidence unexpectedly declined.

Discussion

The results highlight antibiotics as the primary modifiable risk factor for hospital acquired CDI. The declining CDI rates during the pandemic may reflect improved hygiene and infection control

measures through Antimicrobial Stewardship Programs recommendations. The lack of association with proton pump inhibitors use aligns with emerging international findings. The study underscores the need to monitor specific high-risk antimicrobials and to implement tailored antimicrobial stewardship programs.

Conclusions

The study provides critical data for guiding Antimicrobial Stewardship Programs in Costa Rican hospitals and similar healthcare settings. Recommendations include expanding surveillance to primary, secondary and private healthcare facilities, integrating molecular strain typing, and evaluating environmental disinfection practices. Future research should explore the impact of Antimicrobial Stewardship Programs interventions and expand the use of the latest alternatives such as fidaxomicin and continue investigating the potential benefit of fecal transplant.

Resum

Aquesta recerca doctoral representa el primer estudi multicèntric a Amèrica Llatina que analitza les tendències de consum d'antimicrobians i inhibidors de la bomba de protons, i la seva associació amb la infecció nosocomial per *Clostridioides difficile* (ICD) en hospitals de tercer nivell de la Caixa Costarricense de Seguretat Social. L'objectiu general d'aquesta investigació fou cobrir una important bretxa d'evidència científica en aquesta temàtica mitjançant l'avaluació de la incidència, els factors de risc i les característiques clíniques de la ICD, així com les tendències de consum d'antimicrobians durant el període comprès entre els anys 2017 i 2021.

Mètodes

L'estudi constà de tres components: (1) una anàlisi retrospectiva de tendències de consum antimicrobià mitjançant Dosis Diàries Definides (DDD) per cada 100 estades i per cada 100 altes hospitalàries (2017–2021); (2) un estudi ecològic correlacional entre el consum d'antimicrobians i d'inhibidors de la bomba de protons amb la incidència de la ICD, mitjançant anàlisi de sèries temporals i correlació de Spearman; i (3) un estudi de casos i controls amb emparellament per puntuació de propensió per identificar factors de risc en pacients adults hospitalitzats (2019–2021). Es van aplicar regressions logístiques, anàlisi de supervivència de Kaplan-Meier i regressió segmentada de Poisson.

Resultats

- Els antimicrobians més utilitzats durant el període d'estudi van ser la cefotaxima, la vancomicina, l'oxacil·lina i el fluconazole. Tot i la reducció en l'ús de cefalosporines i macròlids, es va observar un augment en el consum de carbapenems, fluoroquinolones i vancomicina.
- Es van identificar 354 casos de ICD i 1172 controls. L'exposició a antibiòtics especialment cefalosporines de tercera generació i clindamicina fou el principal factor de risc. L'ingrés a la UCI, l'estada hospitalària prolongada i les comorbiditats múltiples també s'associaren de manera independent a un major risc.
- No es va trobar una associació estadísticament significativa entre l'ús d' inhibidors de la bomba de protons i la incidència de la ICD. Durant la pandèmia per COVID-19, malgrat l'augment en l'ús d'antimicrobians, la incidència de ICD va disminuir.

Discussió

Els antibiòtics es confirmen com el principal factor de risc modificable per a la ICD. La reducció de casos observada durant la pandèmia podria estar relacionada amb una millora en les mesures d'higiene i control d'infeccions, impulsades per les recomanacions dels Programes d'Optimització de l'Ús d'Antimicrobians (PROA). L'absència d'associació amb els inhibidors de la bomba de protons coincideix amb troballes recents a nivell internacional. Es destaca la importància de monitorar els antimicrobians d'alt risc i d'implementar PROA adaptats als contextos locals.

Conclusions

Aquest estudi aporta evidència clau per orientar els PROA en hospitals de Costa Rica i en entorns sanitaris similars. Es recomana estendre la vigilància als centres d'atenció primària, secundària i al sector privat; incorporar la tipificació molecular de soques i avaluar les pràctiques de desinfecció ambiental. Es proposa investigar l'impacte de les intervencions PROA, ampliar l'ús de les darreres alternatives terapèutiques com la fidaxomicina i continuar investigant el potencial benefici del trasplantament de microbiota fecal.

Resumen

Esta investigación doctoral representa el primer estudio multicéntrico en América Latina que analiza las tendencias de consumo de antimicrobianos e inhibidores de la bomba de protones y su asociación con la infección nosocomial por *Clostridioides difficile* (ICD) en hospitales de tercer nivel de la Caja Costarricense de Seguro Social. El objetivo general de esta investigación fue cerrar una importante brecha de evidencia científica en este tema, mediante la evaluación de la incidencia, factores de riesgo y características clínicas de la ICD, así como las tendencias de uso de antimicrobianos en el periodo comprendido entre 2017 y 2021.

Métodos

El estudio constó de tres componentes: (1) análisis retrospectivo de tendencias de consumo antimicrobiano mediante Dosis Diarias Definidas (DDD) por 100 estancias y por 100 altas hospitalarias (2017–2021); (2) estudio ecológico correlacional entre consumo de antimicrobianos e inhibidores de bomba de protones con la incidencia de ICD mediante análisis de series temporales y correlación de Spearman; y (3) estudio de casos y controles con emparejamiento por puntaje de propensión para identificar factores de riesgo en adultos hospitalizados (2019–2021). Se aplicaron como métodos estadísticos la regresión logística, análisis de supervivencia de Kaplan-Meier y regresión segmentada de Poisson.

Resultados

- Los antimicrobianos más consumidos en el periodo de estudio fueron cefotaxima, vancomicina, oxacilina y fluconazol. Aunque se redujo el uso de cefalosporinas y macrólidos, aumentó el consumo de carbapenémicos, fluoroquinolonas y vancomicina.
- Se identificaron 354 casos de ICD y 1172 controles. La exposición a antibióticos especialmente cefalosporinas de tercera generación y clindamicina fue el principal factor de riesgo. El ingreso a UCI, la estancia hospitalaria prolongada y las comorbilidades múltiples también se asociaron de forma independiente con mayor riesgo.
- No se encontró una asociación estadísticamente significativa entre el uso de IBP y la incidencia de ICD. Durante la pandemia por COVID-19, a pesar del aumento en el uso de antimicrobianos, la incidencia de ICD disminuyó.

Discusión

Los antibióticos se confirman como el principal factor de riesgo modificable para la ICD. La disminución de casos durante la pandemia podría deberse a una mejora en las medidas de higiene y control de infecciones impulsadas por recomendaciones de los Programas de Optimización de Uso de Antimicrobianos (PROA). La falta de asociación con inhibidores de bomba de protones coincide con hallazgos internacionales recientes. Se subraya la importancia de vigilar antimicrobianos de alto riesgo e implementar programas PROA adaptados.

Conclusiones

El estudio aporta evidencia clave para orientar los PROA en hospitales de Costa Rica y entornos similares. Se recomienda extender la vigilancia a centros de atención primaria, secundaria y al sector privado; incorporar tipificación molecular de cepas y evaluar prácticas de desinfección ambiental. Se sugiere investigar el impacto de las intervenciones PROA, ampliar el uso de las últimas alternativas como la fidaxomicina y continuar investigando el potencial beneficio del trasplante de microbiota fecal.

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Introduction

Clostridiodes difficile infection (CDI) has emerged as a critical challenge in the context of healthcare associated infections. Over the past thirty years, its epidemiological profile has undergone a significant transformation, initially noted in Western countries and subsequently observed on a global scale (1).

The organism demonstrated a remarkable capacity for adaptation, presenting ongoing difficulties in clinical management. Infections typically arise in individuals with disrupted intestinal microbiota, most commonly due to prior exposure to antimicrobial treatment (2). Main risk factors include advanced age, immunosuppression and use of broad-spectrum antimicrobials.

A typical case of CDI in a hospitalized patient involves the exposure to *C.difficile* spores, which can germinate, proliferate and release toxins. These toxins are responsible for a spectrum of diseases ranging from mild diarrhea to fulminant colitis and in severe cases, death (3).

The molecular epidemiology of CDI is dynamic over time, across healthcare facilities and geographic regions, it is often influenced by factors such as antimicrobial consumption patterns, pathogenic characteristics of circulating strains, among others. A remarkable example is the emergence of the hypervirulent strain ribotype (RT) 027 (North American pulsed-field gel electrophoresis type) NAP1 in the early 2000s, which has been implicated in severe outbreaks in multiple world regions (4).

Additionally, treatment poses a clinical dilemma, as antimicrobial agents are both a therapy modality and a predisposing factor. Historically, metronidazole and vancomycin were the standard treatments for CDI, however significant advances have been made in the past years. In 2013, fecal microbiota transplantation (FMT) demonstrated efficacy in treating recurrent CDI (rCDI) (5). Moreover, in 2017, bezlotoxumab (a monoclonal antibody targeting CDI) was shown to reduce recurrence rates (6). In the same year, fidaxomicin replaced metronidazole as the first-line therapy for initial CDI episodes (7).

Actually, CDI represents a clinical problem due to its association with increased morbidity, prolonged length of stay (LOS) and elevated healthcare costs. Both surveillance and preventive interventions remain as fundamental strategies in controlling its spread.

1.1 Clostridiodes difficile global and regional epidemiology

In 1893, J.M. T. Finney conducted the earliest anatomic examinations of pseudomembranous colitis (PMC), describing lesions in the intestinal tract of a 22-year-old postoperative patient. By the early 1950s PMC had emerged as a recognized postoperative complication associated with antibiotic therapy, particularly among surgical patients, with reported incidence rates between 14 to 27%. *Staphylococcus aureus* was initially implicated as the causative agent, and oral vancomycin became the standard treatment (8).

Clostridiodes difficile, formerly known as *Clostridium difficile* until 2016, was first detected in 1935 in the faces of healthy neonates (9). It is a strict anaerobe that produces subterminal, non-bulging, elongated spores. Due to its challenging isolation, it was initially named *Bacillus difficilis* (10). Although initially deemed non-pathogenic, the detection of *C.difficile* in patients with PMC-associated diarrhea in 1987 revealed its etiological role in the disease (11).

The prevalence and severity of CDI are shaped by the circulation of specific strains, which in turn are influenced by antimicrobial resistance patterns and local antimicrobial consumption (4). Epidemiological data suggests that up to 3% of healthy adults are asymptomatically colonized by toxigenic *C. difficile*. The rate increases significantly in hospital environments, especially among older adults. Approximately 10% of patients aged over 65 years may already be colonized upon admission. During hospitalization, the colonization rate can rise from 13% to 20% within the first week, reaching up to 50% after four weeks (12).

C.difficile is excreted in faces, and its spores can survive for prolonged periods on dry surfaces. In the healthcare settings, transmission often occurs via contaminated hands of healthcare workers or medical equipment. After ingestion, the spores germinate into vegetative cells; however, in the absence of risk factors, colonized individuals may remain asymptomatic carriers who can still disseminate spores or later develop symptomatic disease (13). As a leading cause of healthcare-associated colitis, *C.difficile* poses a major public health concern due to its easy of transmission and the morbidity and mortality associated with infection (14).

The phylogenetic diversity of *C. difficile* has led to the emergence of various epidemic strains. Polymerase chain reaction (PCR) ribotyping, the gold standard for molecular typing, differentiates strains based on ribosomal Ribonucleic acid (RNA) gene patterns. These RT help distinguish between epidemic (hypervirulent) and non-epidemic strains (Table 1), which vary in prevalence, virulence and antimicrobial resistance. Epidemic RT are identified by their widespread presence across multiple settings and countries (15).

Table 1 Examples of critical *Clostridiodes difficile* ribotypes

CATEGORY	RIBOTYPE	KEY CHARTERISTICS
Epidemic (hypervirulent)	RT027	Produces 16 times more toxin A and 23 times more toxin B than other strains Procuces a binary toxin Highly resistant to fluoroquinolones Common in hospital-acquire CDI
	RT176	Closely related to RT027 Produces a binary toxin Rapid nosocomial spread through Europe
	RT078	Produces toxins A and B and a binary toxin Resistant to fluoroquinolones Common in community-acquired CDI
	RT126	Closely related to RT078 Produces toxins A and B and a binary toxin Frequently resistat erythoromycin, moxifloxacin and tetracycline
	RT 181	Emerging ribotype with high antimicrobial resistance to moxifloxacin, clindamycinand rifampin Shares genetic similarities with RT027
Non-epidemic	RT001/072	Frequently isolated from enviromental sources, such as freshwater sediments Not linked to severe infections
	RT014/020	Common in clinical settings Not typically associated with widespread outbreaks

Adapted from: Molecular Epidemiology of *Clostridioides difficile* Infections in Patients Hospitalized in 2017–2019 at the Central Teaching Hospital of Medical University of Lodz, Central Poland, *Antibiotics*. 2025; 14(3):219 (15)

A substantial rise in CDI incidence and severity has been observed over the past 30 years, largely attributed to the emergence of the hyvervirulent PCR RT 027/toxinotype III, initially identified in Quebec, Canada (1). This strain exhibits high-level fluoroquinolone resistance, enhanced sporulation, elevated toxin production and a mortality rate three times higher than less virulent strains like RT001 or 014 (16). Its introduction into Europe occurred via the United Kingdom, with gradual spread to central and southern European countries (17). The Center for Diseases Control and Prevention (CDC), has classified CDI as an urgent public health threat, requiring immediate action (18).

Cross country comparisons of CDI epidemiology remainw complex due to varying diagnostic criteria, definitions and reporting systems. It is estimated that *C.difficile* is responsible for 25% to 33% of antibiotic associated diarrhea and up to 90% of PMC cases (1). A systematic review covering studies from 2009 to 2019 found a high global incidence, particularly in hospitalized patients. Incidence rates for hospital acquired CDI (HA-CDI) and community-associated CDI (CA-CDI) in the United States were 8.00 and 2.00 per 10 000 patient days respectively. In Canada, HA-CDI was reported at 4.3 per 10 000 patient-days, while CA-CDI were lacking. Among European countries Poland reported the highest incidence (HA-CDI and CA-CDI 6.18 and 1.4 per 10 000 patient day, respectively), while the United Kingdom showed the lowest rates (HA-CDI and CA-CDI 1.99 and 0.56 per 10 000 patient days, respectively) (1,19).

Monitoring CDI epidemiology trends over time has become essential, especially with the emergence of RT such as 012, 023, 027, 014/020, 017,056, 106, which complicate treatment. A recent systematic review and meta-analysis included 59 studies published between 2016 and 2024, from 24 countries found a higher incidence in the hospital settings (5.31 cases per 1000 admissions (95% CI 1.69-2.61) and a higher recurrence rate in CA-CDI (16.22%). Table 2 shows the results of this meta-analysis according CDI rates by each World Health Organization (WHO) region (20).

Table 2 Incidence rate of *Clostridiodes difficile* infection by World Health Organization region

Continent	Incidence of CDI per 1000 Admissions [95% CI]	Incidence of CDI per 10 000 Patients-Days [95% CI]	Incidence of CDI per 10 000 Patients-Days [95% CI]
Eastern Mediterranean	1.13 [0.04-3.64]	1.42 [0.55-2.70]	----
Europe	2.84 [1.76-4.18]	3.57 [2.73-4.52]	36.03 [23.86-50.68]
Latin America	1.69 [1.54-1.84]	3.09 [2.82-3.37]	----
North America	4.85 [4.07-5.70]	6.23 [5.50-7.00]	66.02 [1.05-230.95]
Western Pacific	2.34[1.68-3.11]	3.59 [3.10-4.12]	16.74 [4.30-37.29]
Pooled Incidence Rate	2.56 [2.18-2.96]	3.90 [3.34-4.51]	43.49 [19.51-76.96]

Adapted from: The Global Burden of *Clostridioides difficile* Infections, 2016–2024: A Systematic Review and Meta-Analysis, *Infect. Dis. Rep.* **2025**, *17*(2), 31 (20)

In Latin America, CDI remains underreported due to limited published studies. The first identification of *C.difficile* in the region occurred in Mexico City in 1984, when both toxigenic and non-toxigenic strains were isolated from children treated with antibiotics; although, no link to diarrhea was established at that time (21). In the early 1990s, a Peruvian hospital study found CDI to be rare, with only one case of cytotoxin positivity and negative culture (22). Two decades later, a study in Chile by Gardilcic et al. reported 27 CDI cases over four months in a tertiary hospital, highlighting fluroquinolones as a major risk factor and underscoring the need for antibiotic stewardship (22).

In 2010, Quesada-Gómez et al. published a landmark study in Costa Rica reporting the first identification of the epidemic NAP1/027/ST1 strain in 54% (20/37) of the stool samples from patients with antibiotic-associated diarrhea (23). Peak incidence rates were 29-40 cases/10 000 patient days, with an overall mortality rate of 11.0% (24). In 2012, Hernandez-Rocha et al. identified two NAP1/027/ST1 cases in a Chilean university hospital, confirming the presence and regional dissemination of hypervirulent strains (25). In 2014, López-Ureña et al. reported the first NAP1 strain in Panama; the five isolates were resistant to clindamycin and fluoroquinolones but remained susceptible to metronidazole and vancomycin (26). Subsequently, Camacho-Ortiz et al. described the first NAP1/027/ST1 outbreak in a Mexican tertiary hospital, confirming its emergence in the country (27).

During the COVID-19 pandemic (2020–2021), a modest reduction in global CDI burden was observed compared to the pre-pandemic period. In the United States, a decline in CDI positivity rates was linked to widespread implementation of non-pharmaceutical interventions. However, other studies reported no significant impact of the pandemic on CDI overall. Moreover, some regions with low to middle socio-demographic indices experienced a slight increase in CDI burden during this period (28).

1.2 Pathogenesis and the role of the gut microbiota

C. difficile is a Gram-positive, anaerobic, spore-forming and toxin-producing bacillus capable of colonizing and proliferating in the human gut, particularly following exposure to broad-spectrum antibiotics. These agents disrupt the intestinal microbiota, thereby reducing colonization resistance

and proving an opportunity for *C. difficile* to germinate, disseminate, and cause clinical infection (29).

This microorganism exists in two biological forms: a vegetative state, which is metabolically active and responsible for toxin production and disease manifestation, and a spore form, which plays a pivotal role in transmission. The spore structure comprises protective surface layers, including a coat and an exosporium, which confer resistance to germicidal agents, acid environments, elevated temperatures and antimicrobial agents. These attributes enable *C.diffiicle* to persist for extend periods on various surfaces, facilitating both HA-CDI and CA-CDI (30).

Throughout its life *C. difficile* interacts dynamically with the host gut microbiota. Colonization is closely linked to alterations in the microbiota's composition. The gut ecosystem encompasses diverse microbial domains, including bacteria, archaea, yeasts and viruses. The dominant bacterial phyla are Firmicutes and Bacteroidetes, together accounting for approximately 90% of the microbiota, followed by Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Within the Firmicutes phylum, over 200 genera are identified with *C.difficile* species being highly prevalent. Bacteroidetes are primarily represented by *Bacteroides* and *Prevotella* (Figure 1) (31).

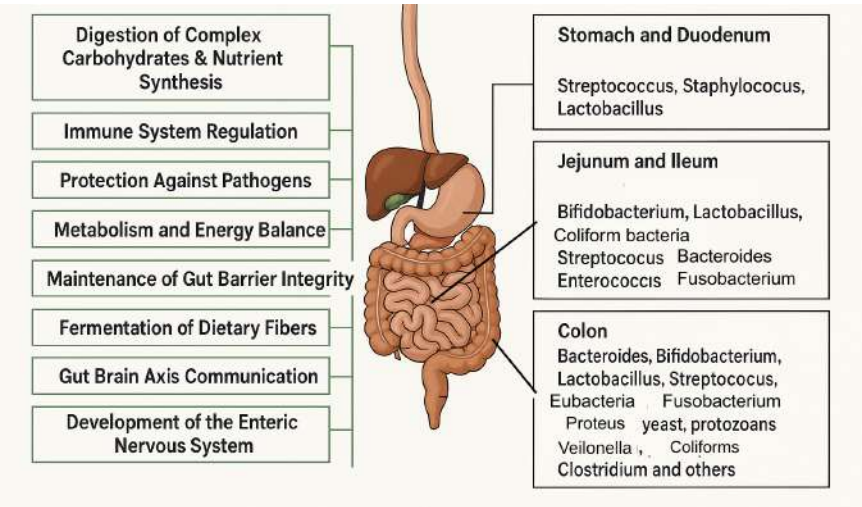


Figure 1 Gut microbiome: distribution of important microorganisms in three different areas of the human gastrointestinal tract

Adapted from: Antibiotics and the gut microbiome: Understanding the impact on human health. Medicine in Microecology 20 (2024) 100106 (32)

In the healthy individuals, this complex microbial community confers protection against *C.difficile* colonization and infection. The adult gut microbiota exhibits three essential features: diversity (characterized by high species richness and functional variety, often associated with health), resilience (the ability to resist and recover from perturbations) and long-term stability at high taxonomic levels, which sustains homeostasis over time (33).

When the gut microbiota is disrupted, for instance by antibiotics, advanced age or immunosuppression, the ingestion of *C. difficile* spores can lead to germination and vegetative outgrowth. Key mechanisms facilitating germination include reduced microbial diversity, decreased competition for nutrients and binding sites, diminished production of bacteriocins, impaired conversion of primary to secondary bile acids, and reduced levels of short-chain fatty acids, which are integral to intestinal homeostasis and anti-inflammatory regulation (29). Germination and adhesion of vegetative *C.difficile* cells to the intestinal epithelium constitute critical steps in disease pathogenesis (12).

Multiple risk factors contribute to intestinal dysbiosis and increased susceptibility to CDI. These include antibiotic therapy, age >65 years, prolonged hospitalization (especially in intensive care units (ICU) or shared hospital rooms), residency in long-term care facilities, immunosuppressive conditions, suppression of gastric acid via proton pump inhibitors (PPI) or histamine 2- receptors antagonist, extended use of elemental diets in enteral nutrition, inflammatory bowel diseases and gastrointestinal surgeries. Each of these factors has been associated with characteristics shifts in microbiota composition and a heightened risk of CDI (Figure 2) (33).

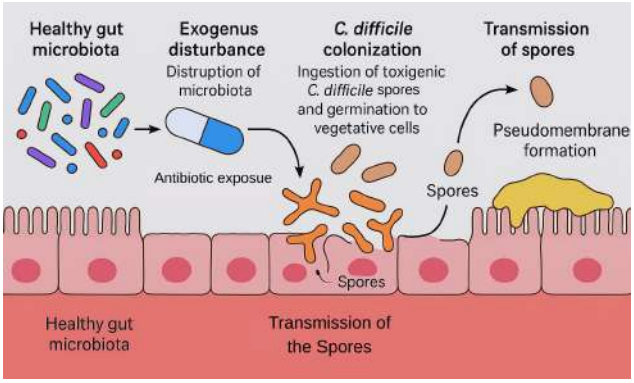


Figure 2. Representation of *Clostridiodes difficile* colonization

Adapted from: Asymptomatic *Clostridium difficile* colonization: epidemiology and clinical implications BMC Infectious Diseases volume 15, Article number: 516 (2015)(34)

Virulence factors for *C.difficile* include surface-layer proteins that mediate strong adherence to the intestinal epithelium and modulate host immune responses. Notably, the bacterium expresses collagen binding proteins that facilitate mucosal adhesion, biofilm formation and evasion of immune defenses (35).

Once colonization is established in the colon, *C.difficile* releases two potent exotoxins Toxin A and Toxin B (TcdA and TcdB) which are considered the principal virulence factors for CDI. These large clostridial toxins (LCTs) are glycosyltransferases composed of four functional domains and are responsible for severe mucosal injury and inflammatory responses (12). TcdA and TcdB bind host cell surface receptors and are internalized via endocytosis. Within the endosome, acidification triggers a conformational change that enables the formation of pores through which the glucosyltransferase domain (GTD) enters the cytosol. The GTD then glucosylates Rho-family GTPases, leading to cytoskeletal disruption, epithelial barrier dysfunction and eventually apoptosis (36).

Some hypervirulent strains, such as PCR RT027, also produce a third virulence factor, *C. difficile* transferase (CDT or binary toxin). CDT is an ADP-ribosyltransferase that binds to host cells receptors, is internalized and depolymerizes actin, exacerbating cellular injury and promoting adherence and colonization (14).

The combined action of these toxins results in hallmark features of CDI- fluid secretion, intense inflammation and mucosal damage. During this process, *C.difficile* also initiates sporulation, allowing transmission to new hosts and perpetuation of the infectious cycle (14). TcdB in particularly, exhibits strong cytotoxic effects, directly damaging the intestinal mucosa. TcdA, while more associated with fluid loss and hemorrhagic necrosis, also contributes to disease severity through is proinflammatory effects (30).

Animal model studies initially suggested that TcdA was the dominat toxin, with TcdB exerting pathogenic effects only in conjuction with TcdA induced tissue injury. However, human colonic tissue models have shown that TcdB alone can elicit severe inflammation and epithelial damage and both toxins can independently trigger CDI symptoms (37,38).

In severe cases, the mucosal injury progress to the formation of characteristics pseudomembranes accumulations of necrotic epithelial cells, neutrophils and fibrin on the intestinal surface, representing one of the classic histopathological features of PMC (Figure 3).

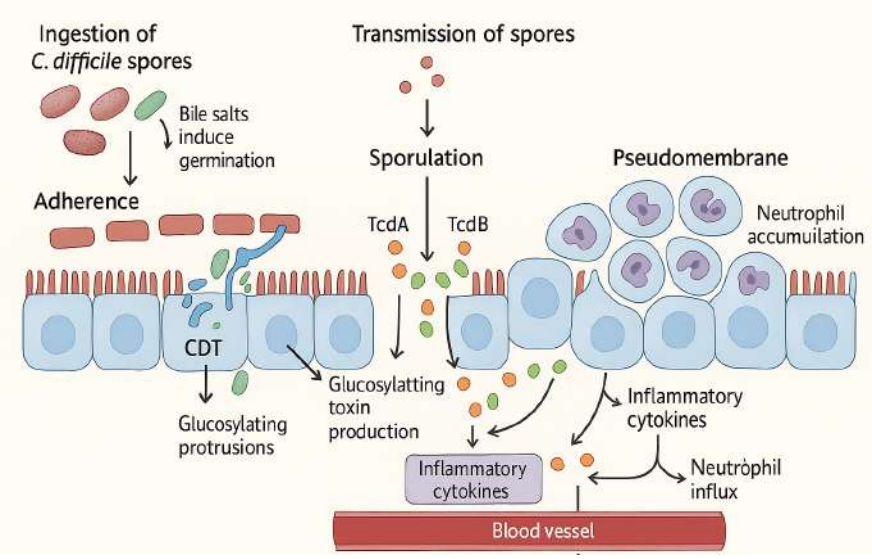


Figure 3. Pathogenesis *Clostridiodes difficile*

After disruption of the gut microbiota, spores of *C.difficile* germinate. Vegetative cells of *C.difficile* colonize the colon, where they release toxins A and B (TcdA and TcdB), this leads to severe inflammation due to massive neutrophil and monocyte recruitment, triggering the opening of epithelial cell junctions and apoptosis. Together, these factors can lead to the formation of a pseudomembrane and the manifestation of disease.

Adapted from: *Clostridium difficile* infection: new developments in epidemiology and pathogenesis Nature Reviews Microbiology volume 7, pages526–536 (2009) (39)

1.3 Risk factors associated with *Clostridiodes difficile* infection

When considering risk factors for CDI, it is important to recognize that they vary depending on whether the concern is a first episode, recurrent diseases or severe/complicated CDI. Host-related factors such as genetics, gut microbiota, age, immunity and comorbidities modulate individual susceptibility. Among these, antibiotic use and advanced age are the most relevant and well-established risk factors. CDI risk factors are commonly categorized into environmental (exposure-related) and host-related factors (Figure 4).

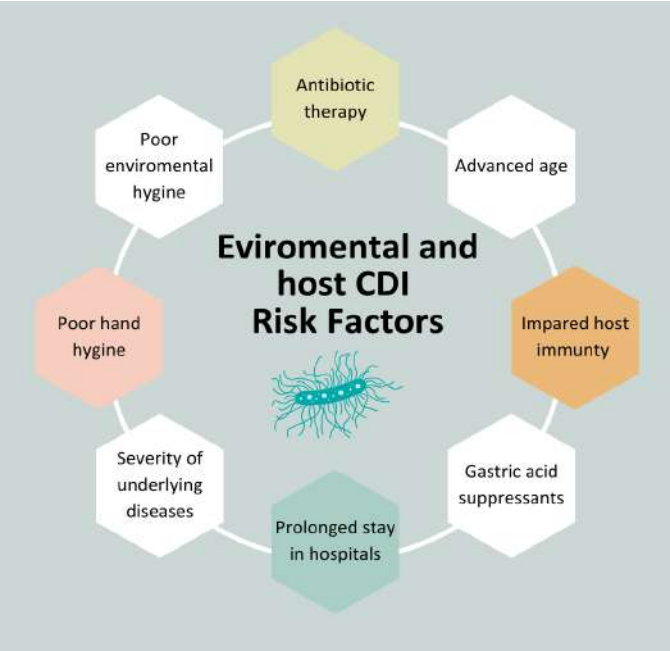


Figure 4. Relevant environmental and host *Clostridiodes difficile* infection risk factors

Original artwork by the thesis author

1.3.1 Environmental risks

As a spore-forming bacterium, *C.difficile* can persist on inanimate surfaces for extended periods. Admission to acute care hospitals, long-term care facilities or rehabilitation centers increases the risk of acquiring *C.difficile*. Risk rises with longer lengths of stay and proximity to symptomatic individuals, both of which heighten exposure to toxigenic strains. Higher levels of environmental contamination are directly associated with *C. difficile* presence on the hands of healthcare personnel. The CDC recommends handwashing with soap and water over alcohol-based sanitizers and strict isolation precautions for patients with active CDI. These practices are essential to reduce transmission between patients and healthcare workers (40,41).

Environmental disinfection using sporicidal agents is critical, as conventional quaternary ammonium compounds used in healthcare are not effective against spores and may even trigger sporulation. Chlorine-based solutions (for example sodium hypochlorite or isocyanuric acid compounds) are sporicidal and have demonstrated efficacy against hypervirulent strains, including NAP1/027 (42,43). It is vital to ensure that all high touch surfaces are cleaned thoroughly. Patients discharged home or managed as outpatients are often advised to clean bathroom surfaces with freshly prepared 10% bleach solutions to minimize the risk of home transmission.

1.3.2 Host-Related Risk Factors

Host-related risk factors play an important role in who becomes infected but also in predicting the likelihood of recurrence. The most relevant factors are described below.

a. Host immunity

There is robust evidence that asymptomatic colonization with *C. difficile* confers protection against progression to active disease through an immune-mediated mechanism. A prospective study among hospitalized patients found that asymptomatic carries had higher IgG levels at colonization compared to patients who later developed diarrhea. Moreover, increased IgM levels targeting toxins A. B and non-toxin antigens were associated with a protective effect. In contrast, patients unable to generate anti-toxin A were 48 times more likely to develop diarrhea (40,44).

Many healthy children and up to 60% of adults have detectable serum IgG and IgA antibodies against toxins A and B, even in the absence of active infection. This suggests that adaptative immunity against *C.difficile* may develop from early-life environmental exposures and persists as a dynamic, host-mediated defense mechanism (34,45).

b. Antibiotic exposure

Antibiotic use is the most important modifiable risk factor for CDI. Antibiotics disrupt the intestinal microbiota, allowing overgrowth of *C.difficile* (46). The extent of CDI risk varies by antibiotic class, with further stratification withing classes. This risk evolves over time in response to changes in strain prevalence and antimicrobial resistance patterns (1).

When *C.difficile* is resistant to the antibiotic to which the patient is exposed, the risk of CDI increases significantly (30,47). Antibiotics reduce microbiota diversity, alter metabolic activity and favor the selection of resistant organisms. The mechanisms by which antibiotics affect gut cells and microbiota are illustrated in Figure 5 (48,49).

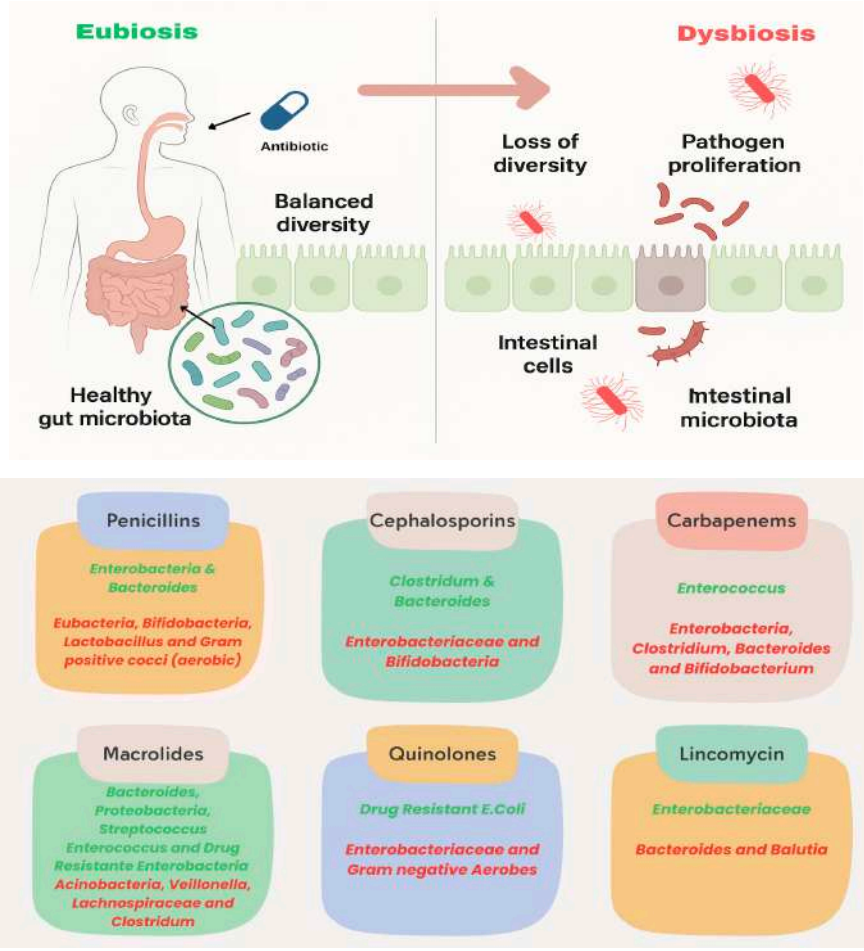


Figure 5. Action of antibiotics on gut cells and microbiota

Organisms cited using green and red color indicates their increasing and decreasing concentration in the presence of the appropriate antibiotics respectively

Adapted from: Antibiotics and the gut microbiome: Understanding the impact on human health. Medicine in Microecology 20 (2024) 100106 (32)

Antibiotics most frequently associated with CDI include ampicillin, amoxicillin, cephalosporins, clindamycin and quinolones. Among these, third generation cephalosporins pose the highest risk due to *C. difficile* intrinsic resistance. The CDI risk tends to decrease from third/fourth, to second to first generation (Figure 6) (1,30).

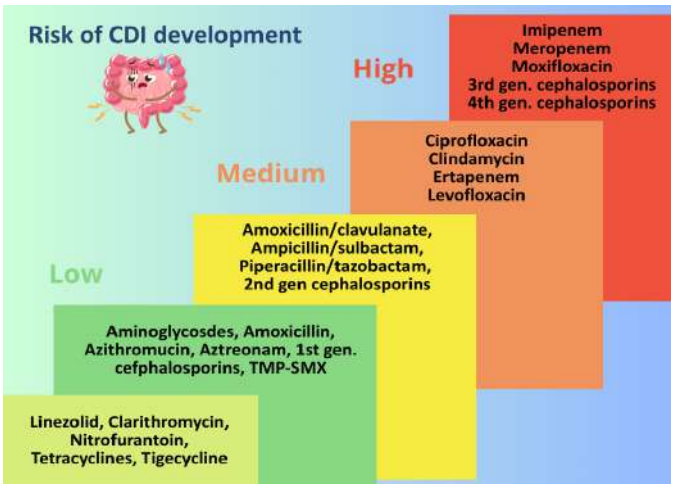


Figure 6. Risk of *Clostridiodes difficile* infection development according to antibiotic classes

Adapted from: *Clostridioides difficile* infection: history, epidemiology, risk factors, prevention, clinical manifestations, treatment, and future options (1)

Duration of antibiotic therapy is also critical: longer courses cause greater and more sustained microbiota disruption. For instance, a 14-day course is associated with a 27% increased CDI risk, compared to 7% with a 7-day course. Even 5-day regimens carry measurable risk. CDI risk peaks during and within the first month after antibiotic use but remains elevated for up to three months post exposure (50,51). Concomitant use of multiple antimicrobials further increases CDI risk (52). Importantly, even single dose prophylactic antibiotics for surgery may increase CDI risk (52).

c. Age

Advanced age is a well-recognized, independent risk factor for incident CDI, rCDI, severe disease and CDI related mortality. Individuals aged ≥ 65 years, particularly those with comorbidities like inflammatory bowel disease, chronic kidney diseases or immunodeficiency are at heightened risk. Aging also reduces gut microbiota diversity, particularly affecting protective species such as bifidobacteria. These microbes produce beneficial metabolites such as short-chain fatty acids and bacteriocins (53).

The relative abundance of bifidobacteria declines with age:

- 60-70% in early life
- 30-40% in early adulthood
- 10% in late adulthood
- 0-5% in the elderly (36).

Additionally, both cellular and humoral immune responses decline with age, further contributing to susceptibility.

d. Gastric acid suppression

Gastric acidity serves as a non-specific barrier against ingested pathogens. Since *C.difficile* transmission occurs via ingestion, the potential link between CDI and gastric acid suppression has been widely explored (54). While *C. difficile* spores can survive gastric acidity, the use of PPIs has been associated with intestinal dysbiosis, which may facilitate colonization. Animal studies show that PPI use may cause epithelial damage, edema, and neutrophil infiltration in colon (55).

However, the exact causal relationship between PPIs and CDI remains unclear. Some meta-analysis report very low-quality evidence for an association and Infectious Diseases Society of America (IDSA) guidelines state that current data are insufficient to recommend discontinuing PPIs solely to prevent CDI. Nonetheless, stewardship initiatives to reassess PPI necessity are encouraged (56,57).

Other well-established risk factors include prolonged hospitalization, inflammatory bowel disease, gastrointestinal surgery, immunosuppression, malignacy, organ transplantation and chronic kidney disease(36).

1.4 Clinical manifestations

1.4.1 Colonic manifestations

a. Asymptomatic carrier:

The acquisition of toxigenic *C. difficile* frequently leads to colonization without clinical illness. Asymptomatic *C. difficile* colonization is the condition where *C. difficile* is detected in the absence of symptoms of infection. This is condition is relatively common in healthcare facilities, with prevalence or incidence rates ranging from 7% to 18% in hospitals and up to 51% in long term care facilities. The number of colonized patients often is higher than symptomatic CDI cases among hospital patients, particularly when disease is endemic. After successful treatment, patients with CDI frequently transition to asymptomatic carriage while continuing to shed spores (58,59).

Moreover, the majority of infants are colonized with *C.difficile* but remain asymptomatic, possibly due to the absence of toxin-binding receptors in the infant gut, as suggested by the common

development of antibodies to *C.difficile* toxins in infants without clinical infection (30). Asymptomatic colonization by toxigenic *C.difficile* in infants appears to stimulates a durable immune response that appears to protect against symptomatic infection later in life (60).

It has been proposed that asymptomatic carries may be protected from disease progression due to their ability to mount an immune response to clostridial toxins. However, these patients potentially act as an infection reservoir and may present a risk to others (34).Despite this, the transmission potential from asymptomatic carriers is lower than of symptomatic patients, and there is no conclusive evidence supporting the extension of contact precautions to *C.difficile* asymptomatic carriages during their hospital stay (1).

b. *Clostridiodes difficile* associated diarrhea

Symptomatic patients develop a clinical presentation that covers a large spectrum of diseases, with diarrhea and colitis being the most common to life-threatening PMC. The incubation period typically ranges from 2 and 3 days but may extend beyond this in some cases. CDI can affect all parts of the colon, but the distal segment is most commonly affected. Most patients with CDI suffer from mild diarrhea and recover spontaneously after 5 to 10 days of antibiotic withdrawing (57).

Diarrhea usually occurs during or shortly after antimicrobial therapy, although it may also develop weeks later. Hospitalized patients with diarrhea occurring >72 hours after admission while on antibiotic therapy are much more likely to have CDI than an infection caused by an alternative enteric pathogen (61). The wide clinical spectrum is likely influenced by host, microbiota and pathogen-related factors. The significant mortality associated with *C.difficile* typically arises from more severe manifestations, including PMC, fulminant colitis, and toxic megacolon (62).

Symptomatic CDI is mediated through the production of toxins that are cytotoxic to epithelial cells of the colon, causing extensive inflammation and epithelial tissue damage to the host (34). Clinical features of CDI, include watery diarrhea with a characteristic foul odor, abdominal pain, fever, nausea and vomiting, weakness and loss of appetite. Diarrhea could persist for days despite the clinical improvement (1).

c. *Clostridiodes difficile* colitis:

Colitis without pseudomembrane formation is the most common clinical manifestation and is a more serious illness, symptoms are life-threatening and include significant dehydration, abdominal distension, hypoalbuminemia with peripheral edema, and subsequent circulatory shock. Severe CDI may cause paralytic ileus that can evolve into toxic megacolon with significant systemic involvement and high mortality risk.

d. Pseudomembranous colitis

PMC can be identified during sigmoidoscopy as raised yellow plaques, a few centimeters in diameter, scattered over the colorectal mucosa. Sometimes these plaques coalesce to form the classical pseudomembrane. Most commonly, the rectosigmoid area is involved in most patients with PMC. But in approximately one-third of the patients, the pseudomembranes are limited to the proximal colon. PMC is the typical manifestation of full-blown C. difficile colitis and is accompanied by more severe symptoms than those observed in nonspecific colitis. In severely ill patients, elevated white blood cell (WBC) count (>20,000/mL) and hypoalbuminemia (serum albumin <3.0 g/dL) may be observed (63).

e. Fulminat colitis:

Fulminant colitis presents with severe lower quadrant or diffuse abdominal pain accompanied by distension and diarrhea. Some patients may have high fever, chills and marked leukocytosis. Fulminant colitis due to *C. difficile* infections occurs in 3% to 8% of patients. It accounts for most of the serious complications including perforation, prolonged ileus, megacolon and death. Patient with toxic megacolon show signs severe toxicity, such as fever, chills, dehydration and leukocytosis (63). In fulminant CDI refractory to medical therapy or with complications (toxic megacolon, perforation with peritonitis, or septic shock), surgical intervention, including hemicolectomy or subtotal colectomy, may be required. Several retrospective cohort studies have found a survival benefit with early surgical intervention, particularly in patients undergoing total colectomy; nevertheless, overall morbidity and mortality in fulminant CDI is very high despite surgery, with mortality rates up to 80% reported (64).

f. Recurrent CDI:

Recurrent CDI is common, affecting approximately 15%-25% of patients after an initial episode, even in the absence of additional risk factors. Recurrence is characterized by the sudden reappearance of diarrhea and other symptoms usually within one week of stopping *C. difficile* treatment**.** In theory subsequent episodes of CDI are usually less severe compared to the first episode, however, if they are temporarily close or if the patient is very frail, they may contribute to unfavorable outcomes. Recurrent CDI may result from persistent disruption of the gut microbiota due to repeated antibiotic exposure allowing *C. difficile* to overgrowth or from an impaired immune response. Evidence suggests that about half of the recurrent CDI cases are due to relapses of infection with the original strain, whereas the other half is caused by re-infection with different strains (1,63).

1.4.2 Extraintestinal manifestations:

Extraintestinal involvement of CDI is uncommon (approximately 0.17%of CDI cases). These manifestations typically include infiltration of the small intestine and bacteremia, visceral or soft tissue abscess formation, infection of implanted prosthetic devices, wound infections, and osteomyelitis are the main reported extraintestinal localizations (65). Since CDI affects the colon in the vast majority of cases, the risk of bacterial and fungal gut translocations is high, with the colon acting as the port of entry. Mortality rate directly due to CDI is estimated at 5%, whereas mortality associated with CDI complications reaches 15–25%, and up to 34% in ICUs. Poor prognostic indicators include advanced age, high leukocytosis, hypoalbuminemia, and high creatinine level. It has also been shown that the first-ever CDI episode increases the overall risk of death (64).

1.4.3 Classification of CDI according to case origin

The designation of case origin is determined by the time and place of the onset of CDI symptoms (66).

Cases of Hospital-associated CDI (HA-CDI) had an onset of symptoms in a hospital on day three or later, following admission to a healthcare facility on day one or they had onset in the community within 4 weeks of discharge from a hospital (67).

Community-associated CDI (CA-CDI) cases have had no discharge from a hospital within the 12 weeks before the onset of symptoms. In addition, their onset of symptoms either took place outside

health care facilities or took place in a healthcare facility on the day of admission or on the following day (67).

If a CDI case has been discharged from a hospital in the 4-12 weeks before the onset of their symptoms, the case origin is “unknown” (66,67).

The designation of a CDI as rCDI is independent of the designation of CDI case origin. Recurrent CDI are individuals who meet the CDI case definition (including return of diarrhea stools with a positive laboratory test) after completion of CDI treatment, who had new onset of symptoms between 2 and 8 weeks after the onset of symptoms from a previous episode of CDI (57,67)

1.4.4 Classification of CDI according to severity

Multiple clinical scoring systems have been proposed to evaluate CDI severity, aiming to identify patients at greater risk for treatment failure, recurrence ICU admission or mortality. There is no widely accepted model to predict CDI severity and usually severe criteria are based on expert opinion and previously identified risk factors in the literature. This section highlights seven models chosen according their prominence in the literature and prior validation in different studies (68). Table 3 provides a comparative overview of these scoring systems, emphasizing their ability to predict severe disease and related outcomes.

a. ATLAS score

A combination of five simple clinical and laboratory values: ATLAS (Age, Treatment with systemic antibiotics, leukocyte count, albumin and serum creatinine), measured at the time of CDI diagnosis, combined into an 11-category scoring system (rank score 0-10), is able to predict treatment response to CDI therapy. The ATLAS score may be useful to stratify CDI patients to evaluate and compare CDI therapies among patient categories (69). A low ATLAS score correlates with higher cure rates (Score >6 is associated with poor responses) in patients with CDI receiving antibiotic treatment (metronidazole, vancomycin or fidaxomicin). However, there are limitations regarding applicability of this tool in clinical practice, although ATLAS score correlates with cure it does not correlate with recurrence (70).

b. *Clostridiodes difficile* Disease (CDD)/Hines VA CDI severity score

The Hines VA CDI severity score was reported to best predict severe CDI with poor outcome. This score included fever, hypotension, elevated white blood cell count and radiological findings on computed tomography scan (thickened colonic wall, colonic dilation or ascites). These factors need to be assessed soon after a positive test for *C difficile* toxin and within three days of therapy commencement. A score of 3 or more indicated severe CDI (71).

c. Zar score

The Zar score assess the severity of CDI and identify patients who may require more treatment interventions, complications and mortality. It is calculated by summing points based on clinical and laboratory factors. The total score ranges from 0 o 6, with higher scores indicating more severe CDI. A score of 2 or more is often used to classify CDI as severe (6,72).

d. Hensgens score

The Hensgens score is a tool to assess the risk of a complicated course of CDI. The score helps clinicians to identify patients at higher risk for complications. The score is calculated based on the combination of patient characteristics and clinical factors; points are assigned based on the presence or absence of certain clinical risk factors. The score ranges from -3 to 8, a higher score indicates a higher risk of complication (73,74)

e. Shivashankar score

The Shivashankar score, developed by Shivashankar in 2014, is a risk assessment tool used to predict severe or complicated CDI outcomes. It helps to identify patients at higher risk for complications like ICU admission, colectomy or death. The score considers factors like age, WBC count, creatinine levels and medication use (75,76).

f. *Clostridium difficile* Severity Score (CDSS)

The CDSS is a clinical predictor rule used to identify patients with severe CDI. It is based on three factors: age, serum creatinine and white blood cell count. The CDSS helps clinicians determine which patients are at higher risk for complications. A score of 3 indicates a high risk of severe CDI (77).

g. IDSA/SHEA and ESCMID severity criteria

CDI are categorized into non-severe, severe and fulminant, according to IDSA/SHEA guidelines (57,66), non-severe CDI is defined as an infection with white blood cell (WBC) ≤15 000 cells/mL and serum creatinine of <1.5 mg/dL. The ESCMID guidelines (78) have a similar WBC count cut-off point but add that creatinine level should be ≤50% above baseline and core temperature ≤38°C at presentation.

IDSA/SHEA defines severe infection as a WBC > 15000 and a serum creatinine of 1.5 mg/dL, while ESCMID stipulate the same WBC as IDSA/SHEA or creatinine level >50% of baseline or core body temperature >38.5°C, with additional factors, such as dilatation of the large intestine, colonic wall thickening or a pericolonical fat standing at imaging (29).

Fulminant CDI is denoted by hypotension or shock, ileus or megacolon according to IDSA/SHEA guidelines (57), while ESCMID guideline define it as the presence of hypotension, septic shock, elevated lactate, ileus, toxic megacolon, bowel perforation or a fulminant course attributed to CDI (78).

Table 3 Comparative table of CDI severity scores

Score	Model type	Score Range	Clinical Criteria	Outcomes Predicted
ATLAS	Point-based	0-10	Age, systemic antibiotic use, WBC, serum creatine, albumin	Clinical response and mortality
Clostridiodes difficile Disease (CDD)	Point based	0-5	Fever, WBC, ileus, abdominal imaging findings	Diarrhea resolution
Zar Score	Point-based	0-6	Age, WBC, albumin, fever, pseudomembranes, ICU admission	Cure, treatment failure, relapse
Hensgens Score	Point-based	-3 to -8	Age, recent abdominal surgery, hypotension, ICU admission, fever	Severe/ Complicated CDI
Shivashankar Score	Regression based	-2.88-0.61	Age, WBC, narcotic use, H2 antagonist or PPI, creatinine	Severe/ Complicated CDI
Clostridium difficile Severity Score (CDSS)	Point based	0-3	Age, WBC, creatinine	Contributable mortality, ICU admission, toxic megacolon/colectomy
IDSA/SHEA and ESCMID severity criteria	Categorical	Non-severe, Severe and Fluminat	WBC count creatinine, presence of shock, ileus, toxic megacolon	Clinical severity classification

Adapted from: Validation of Clinical Risk Models for *Clostridiodes difficile*-Attributable Outcomes Antimicrob Agents Chemother 66:e00676-22(68)

1.5 *Clostridiodes difficile* infection in Special High-Risk Populations

Several patient groups are frequently exposed to healthcare environments and simultaneously affected by underlying conditions that can increase the prevalence, severity or atypical presentation of CDI.

1.5.1 Critically ill patients with CDI:

CDI represents a significant burden in patients admitted to ICUs due to its high prevalence and elevated complication rates. It is estimated that around 11% of episodes of diarrhea in ICU patients are due to CDI. This infection may determine a higher length of ICU and hospital stay, and a higher overall mortality rate that can reach 30%. Observational studies have shown that patients with CDI in the ICUs also had a higher risk for complications such as shock, surgical interventions and complex clinical courses (79).

Severe colitis may present with fever, abdominal pain and distension. Notably, up to 20% of patients with CDI in the ICU can present ileus without diarrhea which complicates the diagnosis of this infection. Analytical findings, such as leukocytosis, bandemia and increased serum lactic dehydrogenase levels may lead to suspicion of CDI in these patients. Up to 20% of these patients may require colectomy or other surgical procedures. Early surgery can reduce mortality and may be more beneficial in patients aged 65 years or more (79).

1. 5.2 Oncological patients with CDI:

Patients with cancer can exhibit CDI rates due to frequent exposure to well-established risk factors, including recurrent hospitalizations, immunosuppression, antibiotic use (both prophylactic and therapeutic purposes), chemotherapy and the use of enteral and parenteral nutrition (80).

Similarly, some chemotherapy drugs, such as platinum-based agents, taxanes and DNA-topoisomerase inhibitors, have been implicated in a higher risk of CDI. However, other studies have failed to find a consistent association between specific chemotherapy agents and CDI incidence (79).

The pathogenesis of chemotherapy-related CDI is not fully understood. One suggested mechanism is chemotherapy-mediated fecal microbiota disruption, which creates an environment that is appropriate for *C. difficile* growth. Other possible mechanisms are inflammatory changes in the

intestinal mucosa, a decrease in C. difficile toxin deterioration and cancer-related immunological changes that could predispose patients to CDI (80).

1.5.3 Transplanted patients:

Transplanted recipients, including those undergoing solid organ transplantation and hematopoietic stem cell transplantation, have significantly elevated risks of CDI compared to the general population. Diarrhea can be a very common complication in both solid organ transplant and hematopoietic stem cell transplant recipients. In addition to infectious causes such as *C.difficile* and cytomegalovirus, massive use of antibiotics (mainly for treatments but also for prophylaxis), long hospital stays, surgery, immunosuppressants, chemotherapy and graft-versus-host disease (81,82). Hematopoietic stem cell transplant recipients are approximately nine time more likely to develop CDI than the general population, with the highest incidence occurring in the early post-transplant period (83). This fact, together with the use of conditioning chemotherapeutic regimens, suggests that *C. difficile* colonization prior to transplant has a relevant role in many of these patients. The frequency of pseudomembranes is low in hematological patients, which may be related to their limited inflammatory response due to immunosuppression (79).

On the other hand, solid organ transplant recipients have up to five times higher risk of CDI than other patients and is related to the frequent exposure to common risk factors for CDI, such as hospital environment, antibiotic and immunosuppressive therapies (79). This group also presents diagnostic difficulties because diarrhea is a very common complication and can be caused by infectious and non-infectious conditions. Immunosuppressive drugs themselves are a frequent cause of non-infectious diarrhea, with *C. difficile*, cytomegalovirus and norovirus being the most frequent infectious causes (82).

1.5.4 Inflammatory bowel disease patients:

Patients with inflammatory bowel disease (IBD) are more frequently diagnosed with CDI than the general population and represents an important burden on patients with IBD because of the greater healthcare costs and increased need for surgical treatment. Detection of this infection could be more difficult in these patients, because symptoms, such as abdominal pain and diarrhea, could be due to an exacerbation of IBD (84).

CDI risk appears to be higher in ulcerative colitis than Crohn's disease, likely due to more extensive colonic involvement. IBD treatments such as corticosteroids and immunomodulators may impair host defense mechanisms, particularly in individuals with compromised integrity (1,85). These patients often require hospitalization and frequently undergo endoscopic and surgical procedures which also increase the risk of CDI (79).

There are no clear recommendations with respect to the maintenance or modification of IBD treatment during CDI. One multicenter retrospective study revealed that patients treated with immunomodulators plus active treatment against CDI more frequently presented in-hospital megacolon, bowel perforation, hemodynamic shock or respiratory failure, and the primary composite outcome was death or colectomy within 3 months of admission (86).

Giving the high rate of asymptomatic *C.difficile* colonization, diagnostic limitations and the risk of severe complications, clinicians must maintain a high index of suspicion for CDI in IBD patients presenting with gastrointestinal symptoms (79).

1.5.5 Renal disease patients:

Patients diagnosed with chronic kidney disease (CKD) are at increased risk of CDI due to frequent hospitalizations, recurrent antibiotic use, gastric acid suppression and altered gut motility, all of which can promote intestinal dysbiosis. Careful antibiotic stewardship is especially important in this population, both to mitigate nephrotoxicity and to reduce CDI risk (1,87).

1.5.6 Pregnant women

Pregnant women are generally considered at low risk of CDI, because pregnant woman are usually young and healthy people. However, pregnancy, peri and the postpartum period put women at an increased risk of developing CDI. Main risk factors are prior antibiotic therapy and hospitalization. Other risk factors recognized are gestational diabetes, cesarean delivery, age $\geq$35 years old, inflammatory bowel diseases, smoking, multiple pregnancies, presence of co-infections during pregnancy, chronic steroid therapy, obstetric complications and prior surgery. CDI in pregnant women can cause systemic manifestations and pose the risk of complications for both the mother and the fetus (79,88).

1.5.7 Pediatric patients:

During the first months of life, colonization by *C. difficile* is common, reaching its peak by the end of the first year of age. Children under 1 year of age are typically excluded from benign defined as CDI due to the high rate of asymptomatic carriers, the absence of toxin A/B receptors in their immature colon and the high prevalence of diarrhea due to other etiologies (89).

Despite high rates of colonization, children appear to be less affected by active infection compared to adults, the reasons are not yet well understood but some hypotheses have been proposed over the years. The first hypothesis concerns the absence of *C.difficile* toxin receptors in infants, additionally age-dependent changes in intestinal microbiota have been proposed as protective factors against active infection, as well as the transplacental transfer of maternal antitoxin antibodies to newborns (90)

Severe complications of CDI, especially fulminant CDI and PMC are not commonly observed in children. The clinical presentation of CDI in these patients consists in most cases of mild to moderate diarrhea. Fortunately, the hypervirulent strain (027) has only been detected infrequently in pediatric patients (79).

1.6 Diagnosis of CDI

The diagnosis of CDI is primarily clinical but must be supported by laboratory testing when necessary, imaging and endoscopic findings. Prompt diagnosis is essential to guide appropriate patient management and to implement infection control measures, particularly in hospital settings

1.6.1 Stool examination:

Stool-based diagnostic testing is central to confirming CDI. According to the Infectious Diseases Society of America (IDSA) guidelines, CDI should be suspected in patients presenting with unexplained, sudden onset of three or more unformed stools within 24 hours (57).

Diagnosis is based on detection of *C. difficile* toxins directly in a stool sample. Laboratories should only process liquid or formless stool samples, Bristol type 6 or type 7 (Figure 7) to prevent testing asymptomatic carriers, except in cases of paralytic ileus, where rectal swabs may be accepted for testing (29,64).

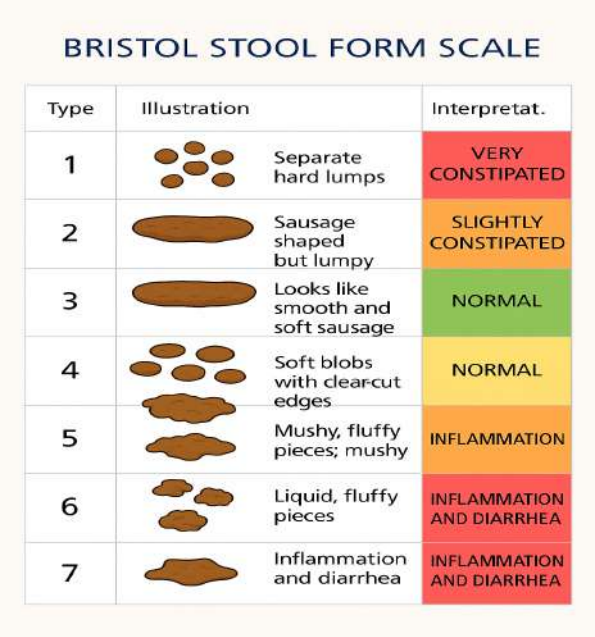


Figure 7. Bristol Stool Scale

Adapted from: Bristol Stool Chart: Types of Poop Web MD (91)

Routine repeat testing for *C. difficile* during the same diarrheal episode is not recommended, except during outbreak investigations. Notably, toxins may still be detectable in stools up to six weeks after treatment and resolution of diarrhea (92).

In pediatric patients, due to the high prevalence of asymptomatic carriage of toxigenic *C. difficile* in infants, testing for CDI should never be routinely recommended for neonates or infants ≤12 months of age with diarrhea. In children ≥2 years of age, *C. difficile* testing is recommended for patients with prolonged or worsening diarrhea and risk factors (underlying inflammatory bowel disease or immunocompromising conditions) or relevant exposures (contact with the healthcare facilities or recent antibiotics) (57).

Testing on the same day of stool collection is highly recommended, if not possible, stool samples should be stores at 4°C for maximum 72 hours. Stool samples should be collected before starting CDI treatment, to avoid false-negative tests (93).

The classical method to detect the free toxin directly from the stool sample by the cell cytotoxicity neutralization assay (CCNA) has historically been the standard for the diagnosis of CDI. The other possibility is to isolate the *C.difficile* strain from the stool sample on selective media in an appropriate anaerobic environment and detect the toxin production of the isolated strain by any toxin detection method, this is referred to as toxigenic culture (TC) (94). CCNA and TC are accurate but no longer used in routine practice because they are not advantageous in terms of time and resources (95).

In most routine laboratories other diagnostics tests are used on stool samples, *C.difficile*-specific antigens detected using enzyme immune assays (EIAs) for glutamate dehydrogenase (GDH) and toxins (TcdA and/or TcdB) and acid amplification tests (NAAT) (95).

GDH is a metabolic enzyme expressed at high levels by all strains of *C. difficile*, both toxigenic and non-toxigenic strains. It shows high sensitivity and a favorable negative predictive value. It is frequently used as a screening test for CDI. On the other hand, NAAT include polymerase chain reaction (PCR), they detect the presence of a toxin encoding genes, confirming the presence of *C. difficile* toxin-producing strain, but it does not necessarily mean that the strain produces any toxins at the moment. As GDH, they have high sensitivity and high negative predictive value (sensitivity 80-100% and specificity 87-99%). The specificity is especially high, reaching 95%, when a negative result is obtained, in this situation, another cause of diarrhea should be considered (64,93).

The IDSA/SHEA and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines stated that no single test is suitable as a stand-alone test confirming CDI. (57,66,78). For this reason, a 2-step testing approach is recommended for the presence of bacterium and its toxins. It uses a combination of EIAs for GDH and TcdA/TcdB, GDH is used as a screening test, if the result is negative, it rules out CDI. A diagnosis of CDI is made if the combined tests are positive. Discordant tests such as a positive GDH and a negative toxin are further clarified with PCR. A negative PCR rules out infection and a positive PCR diagnoses CDI. A flow chart for CDI diagnosis is presented on Figure 8. (29)

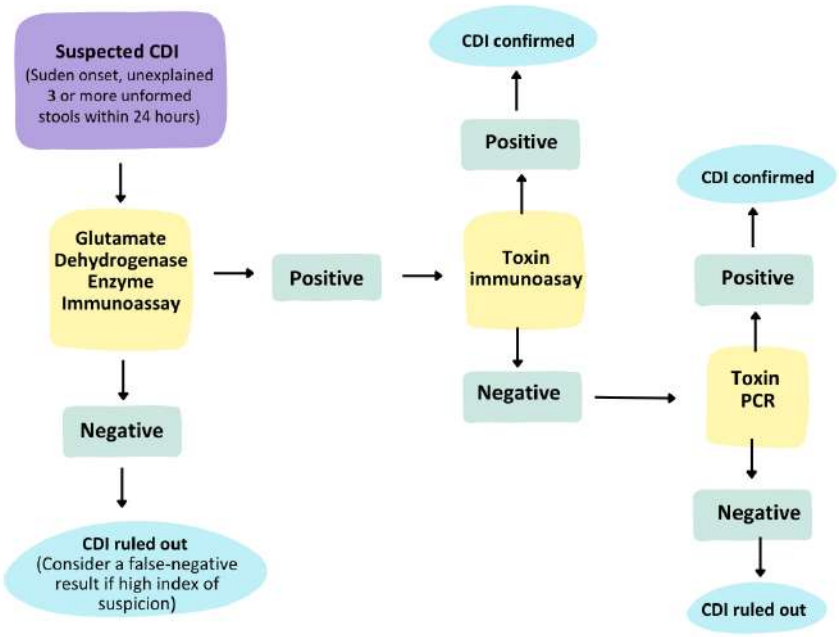


Figure 8. Algorithm for *Clostridiodes difficile* infection diagnosis

Adapted from: *Clostridiodes difficile*: A concise Review of Best Practices and Updates *Journal of Primary Care & Community Health*. 2024;15

1.6.2 **Imaging**

Imaging studies are not routinely needed for CDI diagnosis but play a crucial role in evaluating severe or complicated CDI. Abdominal imaging (X-ray or ultrasound) could reveal distended bowel loops, often with wall thickening. Computed tomography of the abdomen and pelvis with oral and intravenous contrast is useful among patients with severe CDI, helping to evaluate for presence of toxic megacolon, bowel perforation or other finding warranting surgical intervention (76).

1.6.3 Endoscopy

Endoscopic evaluation is useful, but it is not performed in patients with uncomplicated CDI that was confirmed with immunological test. Endoscopy is indicated if diagnostic problems occur, namely a typical CDI presentation with negative *C. difficile* test results, no response to standard course of antibiotics or when an alternative diagnosis is suspected and direct visualization and/or biopsy of the bowel mucosa is needed. When gastrointestinal endoscopy is performed an elevated risk of perforation and bleeding must be taken into account, particularly in cases of fulminant CDI (64).

The typical endoscopic CDI presentation is PMC. Pseudomembranes are lesions of approximately up to 2 cm in diameter, usually yellow or white, irregularly distributed in the colon, and attached to the mucosa. However, not all patients with CDI have pseudomembranes and their absence does not rule out CDI (96). PMC can be caused by several different etiologies, like inflammatory bowel disease, ischemic colitis and other infections like cytomegalovirus (CMV) or enterohemorrhagic *Escherichia coli* O157:H7 (97).

1.7 Treatment of CDI

Antibiotics remain the cornerstone of CDI treatment. Historically, metronidazole, vancomycin and fidaxomicin have been the most commonly used agents. Until 2014, metronidazole was considered the first choice for mild CDI episode, however guidelines have evolved over time, gradually replacing metronidazole with vancomycin in both mild and severe cases. In the latest guidelines updates due to a significant reduction in recurrent rates, fidaxomicin is the preferred option in initial non severe CDI and the first CDI recurrence. Treatment should only be started in patients with CDI symptoms; presence of *C.difficile* toxin without symptoms of infection is not an indication for treatment (66,78,98).

Beyond clinical efficacy, the antibiotic of choice should align with good antimicrobial stewardship principles, prioritizing rapid restoration of the gut microbiota to minimize the risk of recurrence (98).

1.7.1 Pharmacological properties of antibiotics used for treatment of CDI

A comparison of the pharmacodynamic, pharmacokinetic and microbiological properties for oral administration of metronidazole, vancomycin and fidaxomicin is shown in Figure 9.

a. **Metrondazole**: introduced in 1960, is a nitroimidazole antibiotic used as antibacterial and antiprotozoal medication. It is effective against infections caused by susceptible anaerobic organisms such as Bacteroides, Fusobacterium, Peptostreptococcus, *Prevotella* and *Clostridium* (*Clostridiodes*) (99). Metronidazole inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. Oral metronidazole is absorbed almost completely (90%) in the upper gastrointestinal tract and enters the large intestine primarily through secretion across the gut mucosa (98). Although it is not a Food and Drug Administration (FDA)-approved indication, historically, metronidazole has long been considered a first-line therapeutic

option based on the 90–95% success rate documented in early clinical trials. However, surveillance studies have since described an increasing trend in metronidazole minimum inhibitory concentration (MIC) values for common *C. difficile* strains, which may contribute to the increasing number of metronidazole-associated treatment failures observed over the past decade. Additionally, metronidazole can largely affect the gut microbiota because it is active against anaerobic bacteria, including gram-negative anaerobes (100).

b. **Vancomycin**: introduced in 1954, is a glycopeptide antibiotic with bacteriostatic activity that inhibits peptidoglycan biosynthesis in the cell wall in gram-positive bacteria. It is administered mostly intravenously, as it is not absorbed from the intestine. Vancomycin is used for the treatment of complicated skin infections, bloodstream infections, endocarditis, bone and joint infections and meningitis caused by methicillin-resistant *Staphylococcus aureus*. Orally giving as a treatment for severe CDI has the advantage of acting only in the intestine. Nearly all strains of *C. difficile* maintain high susceptibility to vancomycin in vitro, also has the potential to achieve high fecal concentrations. Vancomycin inhibits various aerobic and anaerobic gram-positive bacteria present in gut microbiota. Under high intestinal concentrations can suppress the *Bacteroides/Prevotella* group bacteria (99,100).

c. **Fidaxomicin:** approved by the FDA in 2011, is a narrow-spectrum macrocyclic antibiotic that targets bacterial RNA polymerase, with a bactericidal activity against Clostria belonging to clusters and gram-positive non-spore-forming rods and anaerobic gram-positive cocci. When giving orally it is hardly absorbed from the intestine. Fidaxomicin selectively eradicates pathogenic *C.difficile* with relatively little disruption to the multiple species of the gut microbiota due to its little effect or no activity against gram-negative aerobic and anaerobic bacteria. Fidaxomicin can achieve high fecal concentrations and exhibit a prolonged post-antibiotic effect (98,100). Clinical trials for approval showed that it is not inferior to oral vancomycin; clinical cure was achieved in 77.7% of patients (Vancomycin 67.1%), recurrence of CDI was lower (13.3%) than with oral vancomycin (24.0%) (101).

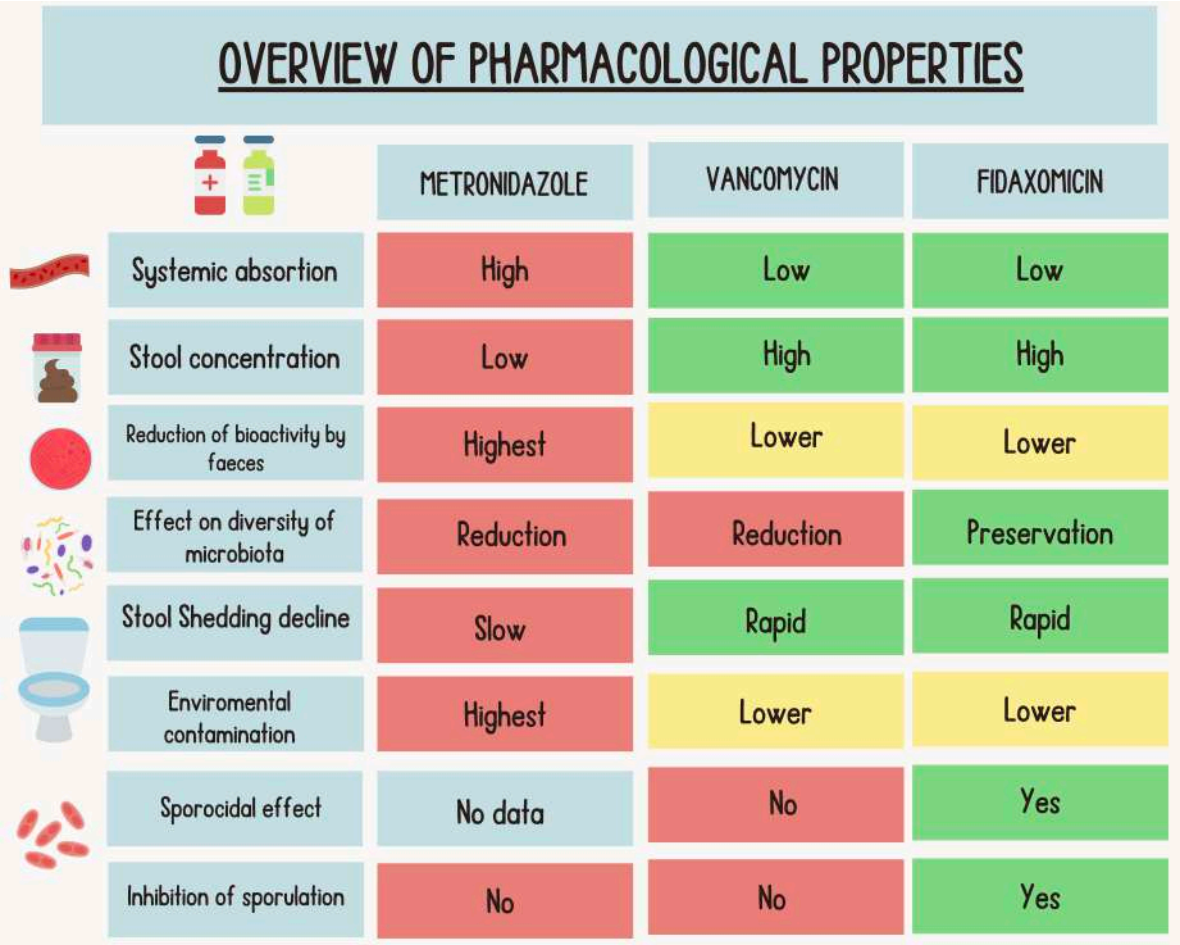


Figure 9 Overview of pharmacological properties for oral administration of metronidazole, vancomycin and fidaxomicin

Adapted from *Clostridioides difficile* infection: are the three currently used antibiotic treatment options equal from pharmacological and microbiological points of view? International Journal of Infectious Diseases Volume 124, November 2022, Pages 118-123

d. **Tygecicline**: is a broad-spectrum protein synthesis inhibitor of the glycylglycine class, active against gram-positive, gram-negative bacteria and anaerobes, including *C. difficile*, *Fusobacterium* spp, *Prevotella* spp, *Porphyromonas* spp and *Bacteriodes fragilis* group. Data confirm that tigecycline still maintains very low resistance rates (1%) against *C. difficile*. For this reason, is a good companion drug in severe/complicated CDI cases especially when bacterial translocation is suspected (1).

Based on a clinical trial that compared vancomycin and metronidazole treatment in 150 patients stratified by disease severity, metronidazole and vancomycin resulted in a clinical cure in 90% and 98% of patients with mild disease and 76% and 97% of patients with severe disease respectively, demonstrating that metronidazole does not perform as favorably as documented in early trials (99).

As a result, an update in 2010 IDSA guidelines for CDI treatment recommended against the use of metronidazole specifically for patients with severe disease due to lower clinical cure rates (100).

Moreover, a second study demonstrated that metronidazole was inferior to vancomycin by showcasing that vancomycin use was associated with a significantly lower 30-day mortality than metronidazole. This favorable response was more evident in patients with severe CDI, in which vancomycin significantly reduced the risk of all-cause 30-day mortality by 20% (100).

Based on this evidence, the IDSA CDI treatment guidelines published in 2021 recommended against metronidazole for all adult patients with CDI and instead recommend vancomycin or fidaxomicin, while metronidazole should only be considered in mild cases when the other two agents are unavailable (66).

1.7.2 Treatment of first episode:

For non-fulminant CDI, treatment with oral vancomycin or oral fidaxomicin have demonstrated similar clinical cure rates. While fidaxomicin and vancomycin have similar efficacy regarding the clinical cure or resolution of acute diarrheal disease, many experts prefer fidaxomicin over vancomycin as it has been associated with the improved sustained resolution of disease, a shorter time to the resolution of diarrhea, and most importantly, a lower recurrence rate. Fidaxomicin has demonstrated a relative risk reduction in recurrence of approximately 40–50% in clinical trials, which included patients with first or second episodes of CDI. For this reason, IDSA guidelines preferentially recommend fidaxomicin for the treatment of the first episode of non-fulminant CDI or for first recurrence (66,100).

Treatment of CDI is based on severity (Figure 10). An initial episode of non-fulminant CDI is treated for 10 days with oral fidaxomicin 200 mg twice daily or oral vancomycin 125 mg 4 times daily. Oral metronidazole 500 mg 3 times daily for 10 to 14 days can be considered if fidaxomicin or vancomycin is unavailable (66,78).

If the patient has risk factors for recurrence including a previous CDI, consider using fidaxomicin 200 mg twice daily for 10 days over vancomycin or using an extended fidaxomicin regimen

(fidaxomicin 200 mg twice daily for 5 days then every other day for up to day 25) and adding one dose of IV bezlotoxumab 10 mg/kg. Oral metronidazole is not an acceptable treatment for severe CDI (29,66).

Bezlotoxumab is a monoclonal antibody against toxin B. A single dose decreases the recurrence rate by 10% in patients at high risk of recurrence. It is cost effective as it prevents relapse and hospitalization in patients >65 years, immunocompromised or with severe CDI (102).

Fulminant CDI is treated orally or via nasogastric tube with vancomycin 500 mg 4 times daily with consideration of adding intravenous metronidazole 500 mg every 8 h. Adding rectal vancomycin 500 mg in 100 mL normal saline every 6 h should be considered if ileus is present. In the absence of supportive data, fidaxomicin is not recommended for the treatment of fulminant CDI (66,103).

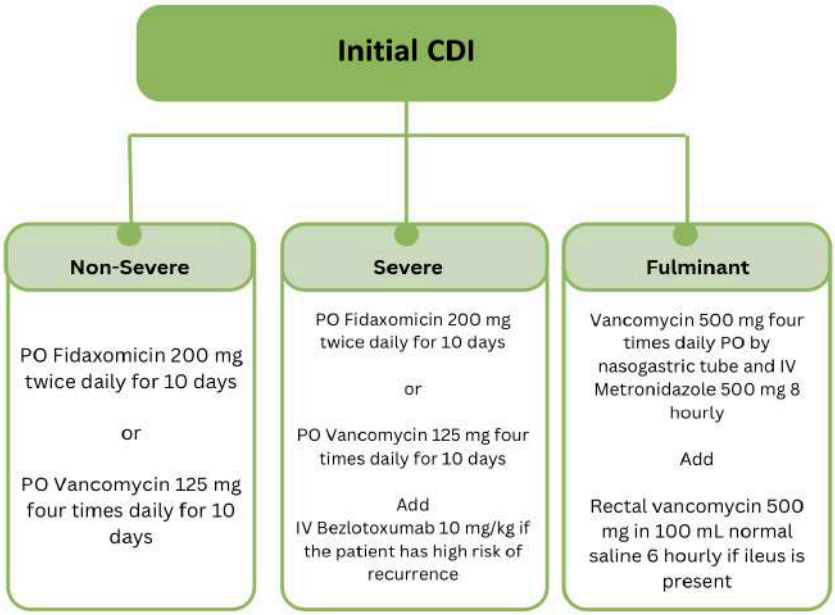


Figure 10. Treatment of initial CDI

Adapted from *Clostridiodes difficile*: a concise review of best practices and updates, Journal of Primary Care & Community Health. 2024;15 (29)

1.7.3 Treatment of recurrence

Recurrent CDI is defined as the return of symptoms of CDI in conjunction with positive laboratory testing for *C. difficile* in a patient who has had an episode of CDI in the preceding 2-8 weeks. Treatment of rCDI is a common clinical challenge, a first recurrence is experienced in 20% of CDI patients who fail to standard of care antibiotics, and nearly 45 to 65% of these patients will

experience multiple recurrences, in addition, the treatment of patients with rCDI is also a challenge because conventional antimicrobials are largely ineffective to obtain a global cure without recurrences (104–106).

Main risk factors for recurrent CDI include advance age, antibiotic se for non- *C.difficile* after CDI diagnosis, gastric acid suppression, hypervirulent strain NAP1/BI/027, severe underlying diseases and/or renal insufficiency, history of previous CDI, previous CDI severity, prolonged hospital stays, lack of adaptive immune responses to toxin A and B (107).

The main international guidelines agree in favoring fidaxomicin over vancomycin for initial episode of recurrent CDI. Tapered/pulsed vancomycin is recommended as an option for recurrent CDI by all the main guidelines. According to IDSA guidelines, bezlotoxumab may be added to the standard of care for CDI recurrence (1).

Initial recurrence is treated using oral vancomycin or fidaxomicin in a standard dose or tapered regimen. If fidaxomicin was used in the first episode, change to extended fidaxomicin 200 mg twice daily for 5 days, followed by 200 mg every other day for days 6 to 25, or vancomycin pulse and taper 125 mg 4 times daily for 10 days, followed by a tapering dose of twice daily, then once daily, then every other day up to 8 weeks. If vancomycin was used in the first episode, change to fidaxomicin, either in a standard regimen of 200 mg twice daily for 10 days or an extended regimen. If metronidazole was used in the initial treatment, change to fidaxomicin or vancomycin standard doses or extended/tapered and pulsed regimens. Adjunctive therapy with a single dose of bezlotoxumab IV at 10 mg/kg may be used with antibiotic treatment (66,78)

Second or subsequent recurrences can be managed with a standard dose or extended fidaxomicin or taper and pulse vancomycin. An alternative approach involves a combination of oral vancomycin (125 mg 4 times daily for 10 days) followed by rifaximin (400 mg 3 times a day for 20 days). Bezlotoxumab can be used in conjunction with antibiotics (66,78).

FMT is recommended for the treatment of a third CDI episode for patients who have relapsed twice despite appropriate antibiotic treatment, and it can be considered in refractory or fulminant CDI (1,66).

1.7.4 Fecal Microbiota Transplantation (FMT) as treatment for CDI

Alterations in the gut microbiota are considered a central factor in the development of CDI. Even short courses of antimicrobial therapy can significantly reduce gut microbial diversity and full recovery may take months. During this period, patients lack their protective barrier and with exposure to spores an infection might develop quickly. Microbiota-based therapies, restore the microbiota, restore proteins and bile acids to levels comparable to a healthy individual and prevent relapse (1,29,108).

Fecal microbiota transplantation (FMT) is a medical intervention involving the transfer of fecal material from a healthy donor to a recipient, with the goal of restoring intestinal microbial balance and enhancing clinical outcomes. FMT has gained recognition due to its effectiveness in treating recurrent *C. difficile* infections and other gastrointestinal disorders. Additionally, it has been studied as an intervention for some inflammatory bowel diseases (106,108).

The proposed mechanisms involve in FMT in the treatment of rCDI may involve restored colonization resistance through bacterial engraftment and modulation of non-bacterial components, direct effect on *C. difficile* through modulation of microbial ecology, inhibition of *C.difficile* growth and germination through bacterial-derived metabolites or modulation of host immune responses and epigenetic responses. The main recognized mechanism contributes to the restoration of gut microbial functionality (106).

According to IDSA/SHEA and ESCMID guidelines, FMT is recommended as a rescue therapy for patients with severe and fulminant CDI refractory to antibiotic treatment and who are not eligible for surgery, also in patients with at least two rCDI episodes to prevent further recurrences, by optimizing the donor, FMT exhibits efficacy of 90-90% (66,78,106).

Numerous randomized controlled trials, systematic reviews and meta-analysis have built evidence for this indication, examining: FMT relative to comparator (placebo, autologous FMT, no intervention, or antibiotics with activity against *C.difficile*), FMT by different routes of administration (enteral tube, oral capsules, colonoscopy or retention enemas) or FMT by different formulations (fresh, frozen, lyophilized), however, on the basis of available evidence, it is difficult to conclude the ideal dosage, route of administration or formulation of FMT and there is no consensus (106).

The improvement of FMT treatment response is influenced by four key factors: donor selection, recipient preparation, graft production, and FMT implementation and management process, among these factors, identifying effective donors is considered the most critical step. FMT response is co-determinate by factors involving the donor, recipient and medical practitioner (see Figure 11) (109).

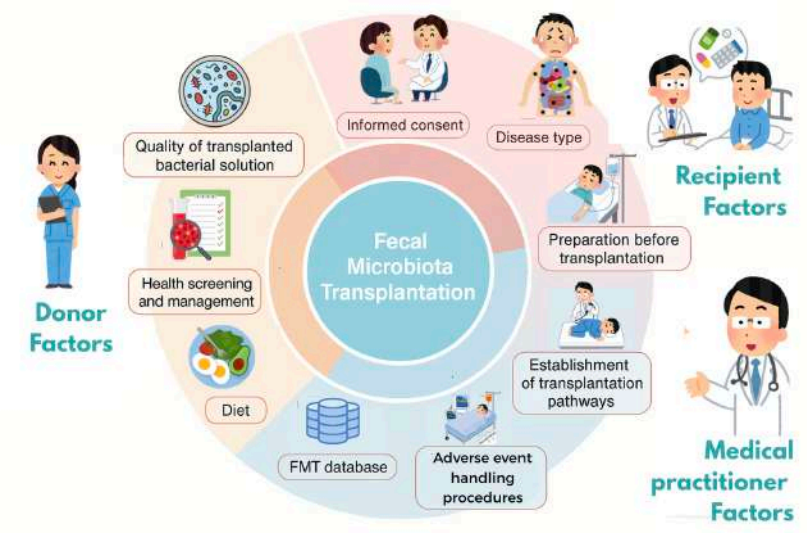


Figure 11. Fecal microbiota transplantation response conditioning factors

Adapted from: Fecal microbiota transplantation in clinical practice: Present controversies and future prospects, Hlife Volume 2, Issue 6, June 2024, Pages 269-283(109)

According to international expert consensus donor screening criteria has three stages. The first stage is medical and drug history screening, the second stage incorporates life history screening, and the third stage adds personal history screening and conducting blood and stool laboratory tests (108). Several new factors, including the diversity and specific composition of both donor and recipient gut microbiota, the immune system and host genetics are thought to be associated with FMT treatment response (109).

In addition, multiple factors related to patient characteristic (presence of inflammatory bowel diseases, CDI-related hospitalization before FTM, inpatient location of FMT and administration of non-CDI antibiotics), procedural pitfalls (poor quality of bowel preparation before colonoscopy) and severity of the CDI may increase the risk of failure up to fourfold after FMT (1).

Manufacturing FMT is poorly standardized, three formulations can be produced, fresh, frozen and lyophilized FMT products, which can be administrated as a slurry or in a capsular form. It is

challenging to produce FMT treatments that have relative consistency to meet regulatory standards as a drug or a biologic, although there do not appear to be significant differences in clinical efficacy in preventing rCDI (110)

FMT can be performed through different procedures, including colonoscopy, nasogastric/nasoduodenal tube or enema or even oral though laboratory-designed frozen oral capsulized preparation (1).

After FMT patients showed increased fecal bacterial diversity, similarly to the healthy donor (Figure 12). Safety concerns are connected with the delivery method (perforation with colonoscopy, aspiration pneumonia with oral administration). Although most side effects are generally mild and self-limiting, severe adverse events, including death and hospitalization have been reported following FMT. Transient diarrhea and abdominal pain occur in ≤10% of FMT procedures (106).

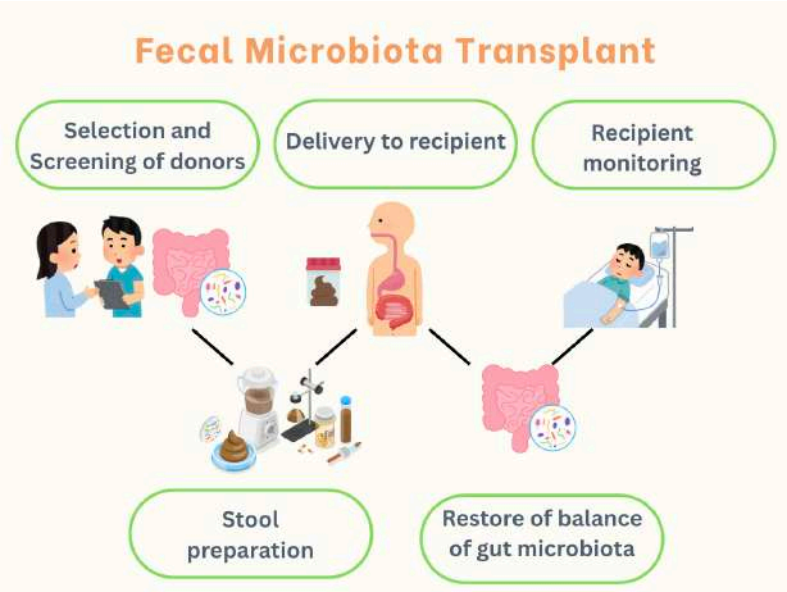


Figure 12 Fecal microbiota transplant procedure

Original artwork by the thesis author

1.7.5 Probiotics

Probiotics are live microorganisms normally found in the intestinal tract, such as bacteria (*Bifidobacteria and Lactobacilli*) and yeasts (*Saccharomyces boulardi*). There are dozens of different species that have the ability of surviving the digestive action of gastric acid, intestinal enzymes and bile salts. They are able to adhere to intestinal cells and begin to colonize them, without giving

immune reactions. Probiotics have beneficial effects: antagonistic action against pathogenic microorganisms, production of antimicrobial substances and protection of the gut against antibiotic associated diarrhea (1,13,111).

The use of probiotics to stop the growth of *C. difficile* has been assessed using various techniques. Several studies show that a few species of *Lactobacillus* and *Bifidobacterium* can reduce the adherence of *C. difficile* to the epithelial cell line and mucus of the intestine. Although there is little evidence on the potential effectiveness of probiotics in CDI treatment, most clinical investigations to date have concentrated on prevention (111,112).

Despite positive reports, the current clinical data are still not sufficient to result in a positive recommendation to use probiotics in the treatment of CDI. The main international guidelines do not support the use of probiotics in any CDI scenario (66,78).

1.8 Infection Control

Infection control is crucial in preventing the spread of *C.difficile* in healthcare settings. Rigorous infection control practices, including proper hand hygiene, contract precautions and environmental cleaning are essential to protect patients and healthcare workers from CDI.

1.8.1 Hand hygiene

Hand hygiene is the foundation of infection prevention. Implementing strict hand hygiene policies for healthcare workers is a key strategy to prevent *C.difficile* cross-infections in healthcare facilities. Alcohol-based hand rubs are not effective in removing *C.difficile* spores, leading to recommendations for hand hygiene preferentially with soap and water before and after caring for a patient with CDI. Handwashing with soap and water is preferred if there is direct contact with feces or an area where fecal contamination is likely (perineal region). Alcohol gel dispenser commonly used in hospitals have even been shown to act as fomites for *C.difficile* (57,113)
Handwashing following the World Health Organization´s 5 moments of hand hygiene is recommended. When staff wear gowns and gloves, they must remove them before leaving the hospital rooms and then perform hand hygiene. The implementation of hand hygiene programs in healthcare settings may have a highly positive impact on improving healthcare workers 'adherence to hand hygiene (113,114).

1.8.2 Isolation and contact precautions

Patients may be exposed to CDI not only because of direct contact with an infected patient but also indirectly by contact with a contaminated environment It is recommended that patients with CDI be isolated in single rooms and or cohorted with other CDI patients to limit patient-to-patient cross transmission (113,115). Furthermore, personal protective equipment (PPE), including gloves and gowns should be worn by providers to limit self-contamination with *C.difficile* and its spores from either the patient or the environment around the patient (57). PPE should be removed and discarded when exiting the patient room before initiating care with other patients (57,78).

The duration of isolation for CDI patients is controversial. There is a widespread agreement that isolation should be continued until the patient is no longer having diarrhea, however, once the patient improves to baseline, there is variability from 24 to 48 h to the duration of stay in the amount of additional time on isolation that is recommended. If feasible in terms of space and staff resources, continuing isolation for 48h after the resolution of diarrhea is recommended to allow for patient hygiene and environmental cleaning (113).

1.8.3 Environmental cleaning

Similarly to hand hygiene, environmental cleaning procedures are among the interventions with the highest theoretical rationale for the control of CDI. Basic rules, such as starting to clean from the cleanest toward the dirties zones of the room should be followed. The most effective intervention, resulting in a 45% to 85% reduction in CDI, include a daily to twice-daily sporicidal disinfection of hospital surfaces, particularly those considered “high touch” like bedrails, door handles and side tables, also shared medical equipment such as blood pressure cuffs, glucometers and portable equipment that travel between rooms (113,114).

During outbreak situations cleaning and/ or the use sporicidal disinfectant agents (1:10 sodium hypochlorite solution) are part of bundled approaches (114). Other no-touch disinfection systems such as continuous ultraviolet germicidal irradiation at the room level have been shown to significantly reduce airborne bacteria, with the potential to lower the incidence of CDI (1).

1.8.4 Antimicrobial stewardship programs

Antimicrobial stewardship involves the development of systematic measures to optimize antimicrobial use, decrease unnecessary antimicrobial exposure, and decrease the emergence and

spread of antibiotic resistance. These measures directly influence CDI epidemiology by improving infection prevention and control measures and promoting appropriate treatment strategies (116).

In fact, CDI incidence has been used as an indirect index of the effectiveness of antimicrobial stewardship programs (ASPs) (Figure 13) (117).

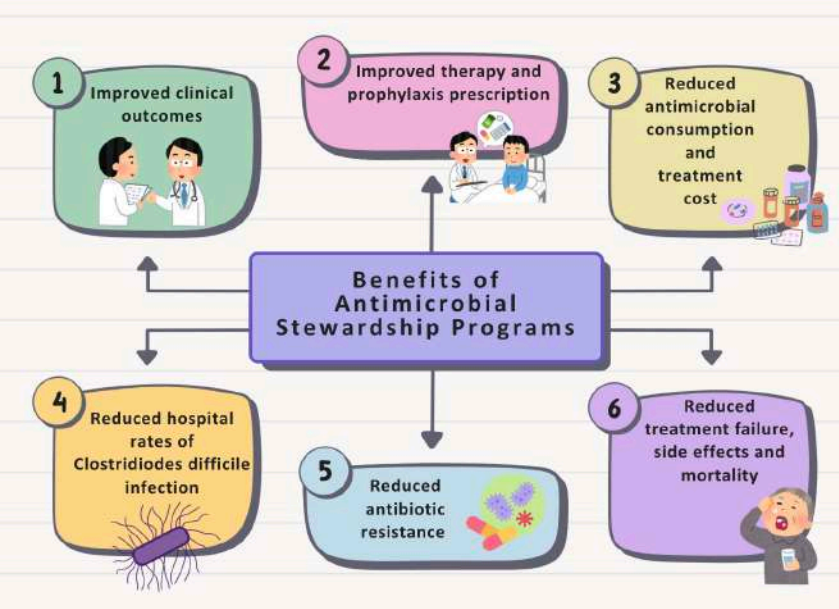


Figure 13 Benefits of Antimicrobial Stewardship Programs

The purpose of ASPs is to improve the use of antimicrobial agents in every clinical aspect

Adapted from: Recommendations for implementing Antimicrobial Stewardship Programs in Latin America and the Caribbean (118)

In addition to optimizing the effectiveness of antimicrobial therapies, ASPs also limit the unnecessary use of antibiotics thus preserving the normal microbiota. Antibiotics to be targeted should be based on the local epidemiology and the *C.difficile* strains present. Restriction of fluroquinolones, clindamycin and cephalosporins (except for surgical antibiotic prophylaxis) should be considered (66). Nonrestrictive polices, such as prospective audit and feedback and implementation of new hospital procedures or guidelines have demonstrated success, often also combined with restrictive strategies (119).

The impact of ASPs on C. difficile risk is documented in multiple systematic reviews that calculate significant pooled risk reductions of 43%–52% in C. difficile infections as a result of ASP interventions (120,121).

International guidelines recommend that every health care facility should have an ASP, including *C.difficile* rates as an indicator outcome along with other antimicrobial resistance pathogens and antimicrobial use quantification (57).

1.9 Surveillance of antimicrobial consumption

Although the association between antimicrobial consumption (AMC) and the risk for developing CDI is well established, the risk profile of antimicrobial groups or individual antimicrobial agents is insufficiently characterized (122). This information could guide ASP interventions to prevent irrational use of antimicrobials or even *C.difficile* outbreaks in the hospital and community settings.

AMC quantification is part of the main objectives of ASP. It involves measuring and analyzing the amount of antimicrobial used, allowing for the identification of trends and areas of improvement in antimicrobial prescribing practices(123,124).

The monitoring of antimicrobial consumption in hospitals uses aggregated local data sources, such as hospital pharmacy dispensing or procurement databases. This allows healthcare facilities to continuously monitor antimicrobial consumption and inform antimicrobial management on a routine basis. To obtain more accurate information on actual antimicrobial use, hospitals need patient-specific information from medical records. Although they provide valuable information on prescribing practices and use, patient-level data for consumption is not always available in many hospitals. Each source of information complement each other and should be employed according to hospital-specific objectives and needs (125).

1.9.1 **The anatomical therapeutic chemical/defined daily dose system**

There is a need for a common system of classification and standard metrics to facilitate comparisons of AMC between health facilities, between countries and between regions. The most used classification system is the anatomical therapeutic chemical (ATC), and the most used measurement metric is the number of defined daily doses (DDDs). In ATC classification, the active substances are divided into different groups according to the organ or system in which they act and their therapeutic pharmacological and chemical properties (126). On the other hand, the DDD is the assumed average maintenance dose per day for a medicine used for its main indication in adults. A DDD is only assigned for drug that already have an ATC code. The DDD is only a technical unit of use, it does not necessarily reflect the recommended or average prescribed dose. There are no separate DDDs for children (127).

Since changes in ATC codes or DDD values are possible, it is important that the ATC/DDD version that has been used for the calculations is noted and reported with the data (125).

The number of DDDs is calculated as follows:

$$\text{Number of DDD} = \frac{\text{Total number of grams of the substance consumed in a defined period of time}}{\text{DDD value of the substance in grams assigned by the WHO}}$$

The number of DDD provides a measure of extend of use, however for comparative purposes these data are usually adjusted for population size or population group or hospital activity depending on the level of data desegregated (128).

Standardization by hospital activity data allows the comparison of data from different time periods and from different hospitals or hospital units. Information on hospital structure and hospital activity data is normally provided by the hospital administration. A selected hospital activities description is provided bellow(125):

- **Patient days (PD)**: the sum of patient days for a defined period of time
- **Bed days (BD)**: sum of bed days of a defined period of time in which the unite was active
- **Occupied bed days (OBD)**: sum of occupied bed days in a defined period of time
- **Admissions (ADM)**: sum of patients admitted in a defined period of time
- **Discharges (DIS)**: sum of patients discharged in a defined period of time

At a hospital level, the most frequently used denominators are: 100 or 1000 OBD and 100 or 1000 ADM (or DIS), while at community level, the most frequently used unit is DDD/1000 inhabitants (124). Calculation of DDD/100 OBD and DDD/100 DIS follows the next formula:

$$\text{Consumption density} = \frac{\text{Number of DDD for time period multiplied by 100}}{\text{Quantity (number) of hospital activity indicator (OBD or DIS)}}$$

The indicator DDD/100 OBD expresses hospital exposure to antibiotics, but it does not describe the density of employment, since it does not provide information on the number and proportion of patients treated with antimicrobials, while the use of DDD/100 DIS provides information on

exposure per patient and helps to interpret consumption trends over time as they show changes in hospital activity. Both measurement units are complementary to interpretate AMC in hospital settings (129).

1.9.2 Other unit of measurement of antimicrobial consumption

Even though WHO ATC/DDD methodology allow the comparison of AMC between different setting and between different drugs, they do not reflect the actual dosage used for individual patients or specific patient populations and their use is not recommended in children (130). For this reason, other AMC indicators like days of therapy (DOTs), length of therapy (LOTs) and prescribed daily dose (PDDs) have been proposed (131).

- **Days of therapy (DOTS)** are defined as the number of days that a patient receives antibiotics regardless of the dose. When a patient receives more than one antibiotic, more than one DOT may be counted

- The **length of therapy (LOT**) is the number of days that a patient receives systemic antimicrobial agents, irrespective of the number of different antibiotics.

- **Prescribed daily dose (PDD)** is derived from actually prescribed/administrated dose to a patient. It is the average dose prescribed according to a representative sample of prescriptions. The PDD will give the average daily amount of a drug that is actually prescribed.

The monitoring of antimicrobial consumption is usually presented as a report that records the periodicity of measurement, the healthcare setting studied and the level of data aggregation. As part of WHO essential medicines list, in 2017 it was introduced a new categorization of antibiotics to guide prescriptions and treatment. The categorization is called AWaRE and is based on a methodological approach that takes into consideration treatment guidelines and infectious diseases.

1.9.3 AWaRe classification of antibiotics for evaluation and monitoring use

The WHO's AWaRe classification of antibiotics (Access, Watch, Reserve) is a tool to promote appropriate antibiotic use and curb antimicrobial resistance. It categorizes antibiotics based on their spectrum of activity, potential for resistance, and intended use, guiding clinicians on when to use which antibiotics. The Access group contains first-choice, narrow-spectrum antibiotics, while the Watch group includes broader-spectrum antibiotics used more cautiously. The Reserve group is reserved for last-resort situations against multidrug-resistant infections (127,132).

The overall goal is to reduce the use of Watch and Reserve group antibiotics and to increase the relative use and the availability of Access group antibiotics where needed. The concept of AWaRe combined with a simple traffic light system used for graphical presentation, may help to raise awareness and understanding of the concept of broad-spectrum and narrow-spectrum antibiotics (125). Figure 14 includes a selection of antibiotics according to the AWaRe classification (133)

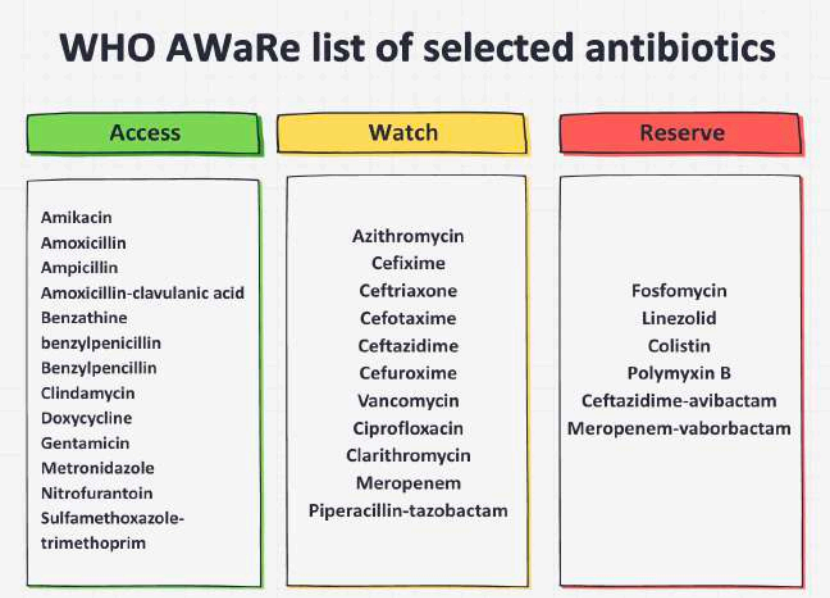


Figure 14 WHO AWaRe classification for antibiotics Adapted from Encouraging AWaRe-ness and discouraging inappropriate antibiotic use—the new 2019 Essential Medicines List becomes a global antibiotic stewardship tool The Lancet Infectious Diseases, Volume 19, Issue 12, 1278 – 1280 (133)

1.10 Overview of Costa Rican Public Healthcare System

Costa Rica is an upper-middle-income country in Central America, with a population of 5 million, mostly urban and concentrated around the capital, 72% live in urban areas and 62% in the metropolitan region (134).

The foundation of Costa Rica´s healthcare system lies in tis social security institution Caja Costarricense de Seguro Social (CCSS) created in 1941. It emerged from a social agreement between the government, employers and workers to provide universal access to healthcare. With a coverage of nearly 95% of the population, including vulnerable groups such as the elderly and low-income individuals, is ranked among one of the best Latin America health systems with health outcomes comparable to those of high-income nations, with life expectancy exceeding 80 years and low infant and maternal mortality rates (135)

The Costa Rican public health care system is structured into three integrated levels of care with a total of 1000 primary care attention centers, 29 hospitals: 3 national (located in the metropolitan area), 6 specialized, 8 regional, and 12 peripherals (136):

- **Primary Care**: Delivered through EBAIS (Equipos Básicos de Atención Integral en Salud), which are community-based multidisciplinary teams offering preventive care, chronic disease follow-up, maternal-child care, and vaccinations**.** These teams are supported by shared staff, including social workers, nurses, nutritionists, pharmacists, microbiologists, and medical records technicians
- **Secondary Care**: Provided in regional hospitals**,** covering general medical and surgical services, diagnostic imaging, and some specialty consultations.
- **Tertiary Care**: Offered by high-complexity national hospitals, which manage specialized procedures**,** transplants, and complex surgeries.

A critical component of the Costa Rican public healthcare system is the "List of official medicines (LOM)**.** The LOM is a formulary of approved medications that the CCSS provides free of charge to insured patients, based on scientific evidence, cost-effectiveness, and national epidemiological needs (137)**.**

Implications and Benefits of the LOM:

- Equity and access: Guarantees universal access to essential and high-priority medications, regardless of patients' income or location.
- Rational use of medicines: Encourages evidence-based prescribing and limits unnecessary or inappropriate use.
- Budget control: Helps the CCSS optimize pharmaceutical spending by prioritizing cost-effective therapies.
- Periodic updates: The LOM is reviewed and updated regularly by a technical committee of experts to include new treatments and generic alternatives based on updated evidence and public health priorities.

1.11 Project justification

CDI is a significant healthcare-associated condition, linked to increased morbidity, extended hospital stays, and substantial healthcare costs. Its development is strongly associated with the use of specific medications, particularly antimicrobials and proton pump inhibitors (PPIs) which are considered the most relevant modifiable risk factors.

In Costa Rica, scientific evidence at population and ecological level regarding the relationship between antibiotic use and PPIs with nosocomial CDI is scarce. This gap limited the ability of ASP, Infection Control Units, Pharmacotherapy Committees, and Pharmacy Services to make data-driven decisions to mitigate the incidence of this infection.

This work is guided by the hypothesis that the exposure of certain families of antibiotics or specific molecules such as third generation cephalosporins, clindamycin and carbapenems and PPIs may be related to a greater risk of generating hospital acquired CDI.

The research conducted addressed this critical evidence gap by analyzing the epidemiological and clinical characteristics of nosocomial CDI, along with patterns of antimicrobial and PPI consumption, in tertiary-level hospitals within Costa Rica's Social Security system (CCSS). The study focused on identifying risk factors associated with CDI, particularly medication use, over the period 2019–2021,

while also assessing medication consumption trends from 2017 to 2021 using Defined Daily Doses (DDD) per 100 bed-days and per 1,000 discharges and correlating them with CDI incidence.

Furthermore, the research considered the potential impact of the COVID-19 pandemic on medication use trends, which may have influenced the epidemiology of CDI in recent years.

By generating robust, context-specific data, this study provides valuable insights into which antimicrobials, antimicrobial groups, present the greatest risk for nosocomial CDI in the Costa Rican hospital setting. These findings offer critical evidence to support decision-making by ASP teams and institutional governance bodies, aiming to improve medication management, reduce infection rates, and contain the clinical and economic burden of CDI within the public health system.

2

Hypothesis and Objectives

Hypothesis and Objectives

The **hypothesis** that was put forward before the completion of this doctoral thesis is that in general tertiary care hospitals of the Social Security in Costa Rica (CCSS), the use of antimicrobials (such as third-generation cephalosporins, clindamycin, and carbapenems) and PPIs are the most important risk factor for the development of nosocomial CDI. Furthermore, the COVID-19 pandemic has led to an increase in in-hospital use of antimicrobials and PPIs, increasing the risk of nosocomial (hospital acquired) CDI.

The **general objective** of this doctoral thesis was to analyze the incidence, clinical characteristics, and risk factors for the development of nosocomial CDI in patients admitted to general tertiary care hospitals of the Social Security in Costa Rica from 2019 to 2021, as well as the trends in antimicrobial and PPI use and its association with the nosocomial CDI rate in these hospitals from 2017 to 2021.

To meet this objective, two studies and an individual analysis were designed with the following specific objectives

Individual analysis:

To determinate the monthly incidence rates of nosocomial CDI, epidemiological and clinical characteristics of the population in study throughout the period 2019 to 2021.

To explore the association between the epidemiological and clinical characteristics of the population in study and the risk of nosocomial CDI throughout the period 2019 to 2021.

First study:

To describe and analyze antimicrobial consumption trends in Social Security tertiary care hospitals across Costa Rica and throughout the period of 2017-2021, using both DDD/100 bed days and DDD/100 discharges as measurements of hospital activity.

Second study:

To investigate the association between antimicrobials and PPI consumption with nosocomial CDI in Social Security tertiary care hospitals across Costa Rica and throughout the period of 2017-2021.

3

Methods

The analysis of the incidence, clinical characteristics, and risk factors for the development of nosocomial CDI in patients admitted to general tertiary care hospitals of the Social Security in Costa Rica from 2019 to 2021, as well as identification of the trends in antimicrobial and PPI consumption and its relationship with the nosocomial CDI rate in these hospitals from 2017 to 2021 were performed through three studies, two of three studies have been properly published.

Hospital setting: these studies were performed at general tertiary care hospitals of the Social Security in Costa Rica, according to the available data, two out of the three hospitals in this group were include: Hospital San Juan de Dios (635 beds) and Hospital México (466 beds). According to Social Security statistics, these hospitals are academic centers with the highest complexity level of health attention, that provide care for approximately 85% of the Costa Rican population, in intensive care units, medical and surgical services. Study protocol was approved by the Costa Rican Social Security Ethics Committee: CEC-CENTRAL-CCSS, Protocol #R022-SABI-00319.

A detailed description of the specific methodology of each study is provided below.

3.1 Population-based study "**Antimicrobial, PPIs exposure and risk factors for hospital-acquired *Clostridiodes difficile* infection: A propensity score matched study**"

3.1.1 Study design

This retrospective observational case-control study was conducted from January 1st, 2019, to December 31st, 2021. All patients with HA-CDI were identified according to Infectious Diseases Unit and Clinical Laboratory records.

3.1.2 Patients and variables

All patients admitted to an inpatient medica, surgical or intensive care unit at the participating hospitals in the period spanning January 1st, 2019, and December 31st, 2021, with a hospital stay of three days (72 hours) or more. Admissions from emergency services, pediatric hospital wards as well as any units that do not generate discharges or occupied bed days were excluded from the data collection process.

Included cases had the diagnostic characteristics described in the local guidelines for the management of the CDI that corresponds with the definition of HA-CDI described in the IDSA/SHEA guidelines: onset of watery diarrhea or unformed stools (type 5 to 7 on the Bristol scale), with three

or more episodes in the last 24 hours of hospitalization, with a positive laboratory test of GHD antigen, toxin A and/or B and PCR molecular testing if necessary. Patients with evidence of PMC diagnosed by endoscopic examination, surgery or histopathology were also included. Also, patients with nosocomial rCDI were identified, a recurrence is defined as the presence of diarrhea with a new positive test within two to eight weeks after the first episode (determinate by the initial laboratory test date, pathological or endoscopic diagnosis) proving that there was an asymptomatic period between both episodes.

Controls were identified with all patients admitted at the participating hospitals in the same period of study with 3 days or more of hospital stay that presented watery diarrhea or unformed stools (type 5 to 7 on the Bristol scale), with three or more episodes in the last 24 hours of hospitalization that presented negative laboratory test for HA-CDI.

Patients with a clinical history of watery diarrhea or unformed stools without laboratory, endoscopic or pathological tests, patients with diagnosis of CA-CDI or occupational CDI were excluded.

Data recorded were collected form clinical the electronic health record system (Unique Digital Health Record, EDUS form its acronym in Spanish) implemented in Costa Rica, which integrates comprehensive real-time access to clinical data (medical history, prescriptions, laboratory test results).

An anonymized form was previously designed for data collection. Epidemiological data included age, sex, hospitalization ward (medica, surgical or ICU), previous medical admission within 8 weeks, underlying diseases and their severity according to the Charlson Comorbidity Index Score, use of immunosuppressive agents, inflammatory bowel disease, COVID-19, septic shock and presence of other concomitant infectious diseases, length of stay, use of antimicrobials, number of antimicrobials used, use of PPIs (oral and/or intravenous) and in-hospital death. For cases variables such as episode severity, treatment received, and clinical outcomes (in hospital death and CDI related death) were also included. A total of three controls for each case were collected.

3.1.3 Statistical analysis

Baseline categorical variables were presented as percentages and were compared using X^2 or Fisher exact test when appropriate. Continuous variables were described as mean and were analyzed by t-test.

Due to imbalances in the baseline characteristics of the cases and controls, a propensity score matching was performed to reduce potential bias. This analysis was performed through logistic

regression and using a one to one nearest-neighbor matching without replacement, with treatment with ICD as the dependent variable. Variables identified by a literature search as previously associated with CDI like age, diseases and their severity (Charlson comorbidity score), inflammatory bowel diseases and use of immunosuppressive agents were included as independent variables. The caliper was set to a width equal to 0.2.

Also, a risk factor analysis was conducted, adjusted risk of CDI with antibiotics and PPIs was identified using Odds ratio (95% IC), additionally a univariate and multivariable logistic regression models for epidemiological and clinical factors (excluding antimicrobials and PPIs) were performed. Finally, an all-cause and disease-specific mortality study was conducted with the cases in the matched cohort. CDI-attributable mortality and all-cause mortality were analyzed with Gray test and Kaplan Meir graphic. $P<0.05$ was considered to indicate statistical significance in all analyses. Statistical analysis was performed using SAS Studio OnDemand for Academics (SAS Institute Inc., Cary NC, USA) Version 3.81 (Edition Enterprise)

3.2 First study: *"Trends in Antimicrobial Consumption in Tertiary Care Hospitals in Costa Rica from 2017 to 2021: A comparative analysis for defined daily doses per 100 bed days and per 100 discharges"(138)*

3.2.1. Setting and Study Design

Retrospective observational study of antimicrobial consumption in tertiary care hospitals of the social security in Costa Rica

3.2.2 Data Collection

Data were collected monthly, starting from January 2017 and ending in December 2021, with the support of the pharmacy department software "Integrated Pharmacy System" (SIFA). Sixty-one antimicrobials for systemic use (J01, J02 and J05), according to the WHO-ATC classification, representing 46 different active principles, were included in the antimicrobial consumption quantification. Pediatric hospital wards, as well as units that do not generate high rates of discharge or occupied bed days (e.g., emergency services) were excluded from the data collection process. A description of the AMC per group and per specific antibiotic was included. Global consumption trends for antimicrobials groups and according to the WHO AWaRe classification were calculated and expressed in DDD/100 bed days and DDD/100 discharges, employing the WHO ATC-DDD Index

2023 for all five years as the assumed average maintenance dose per day for a drug used for its main indication adults. Before starting the study, responsible pharmacists were trained in WHO ATC-DDD calculation and the usage of the DDD Excel Calculator to guarantee a homogeneous collection of data.

3.2.3. Statistics Analysis

Trends in antimicrobial consumption were analyzed using a simple linear regression model to determine potential differences in antimicrobial usage throughout the period of the study. Simple linear regression was used to determine whether there was a significant difference between the calculated coefficient and zero.

The equation for the simple linear regression is

$$Y = \beta 0 + \beta 1X + \epsilon$$

where Y: antimicrobial consumption rate in DDD/100 bed days or DDD/100 discharges (dependent variable), β0: the intercept (the estimated antimicrobial consumption when X = 0, or the starting point), β1: the slope (the estimated change in antimicrobial consumption per year), X: the year or time (independent variable), and ϵ: the error term (captures the variability not explained by the linear relationship) If β1 (the slope) is positive, it suggests an increasing trend in antimicrobial consumption over the 5-year period; if β1 is negative, it indicates a decreasing trend; and if β1 is close to zero and not statistically significant, it suggests no significant trend in consumption over time.

The comparison of DDD, patient days, discharges and average bed days between 2017 and 2021 was carried out using the Wilcoxon signed-rank test. Additionally, the average annual percent change (AAPC), with 95 percent confidence intervals, for different classes of antibiotics was incorporated into the analysis to measure the average percentage change in AMC over time, combining the year-to-year fluctuations into a single value. p values of <0.05 were considered statistically significant. Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond,WA, USA) and SAS Studio OnDemand for Academics (SAS Institute Inc., Cary, NC, USA).

3.3 **Second study** *"Association between antimicrobials and Pump Proton Inhibitors consumption with the incidence of nosocomial Clostridiodes difficile infection in high complexity hospitals in Costa Rica"(139)*

3.3.1. Study Design and Population

The ecological study conducted involved the examination of antimicrobial and PPIs consumption, correlated with nosocomial CDI incidence, coupled with time-series analysis. Patients with nosocomial CDI admitted to participating General Tertiary Care Hospitals from the Costa Rican Social Security (Hospital San Juan de Dios and Hospital Mexico) between January 2017 and December 2021 were included in the analysis. Hospitals were selected based on data availability for antimicrobial and PPIs use, nosocomial CDI rates, and presence of AMS programs. Excluded from the data collection process were pediatric hospital wards, as well as any units that do not generate high discharge rates or occupied bed days (e.g., emergency services). The use of antibiotics in Costa Rican Social Security is regulated by an institutional medicines policy, based on the World Health Organization List of Essential Medicines.

3.3.2 Statistical Analysis

First, as a method of descriptive analysis, annual rates of nosocomial CDI and annual antimicrobial and PPIs consumption trends were described. Changes in antimicrobial consumption during the study period were evaluated using a linear regression test. The AAPC, with 95 percent confidence intervals, for different antimicrobials and PPIs was incorporated into the analysis to measure the average percentage change in antimicrobial consumption over time, thus combining the year-to-year fluctuations into a single value. The statistical analysis was conducted in two main stages: correlation analysis and interrupted time series analysis using Poisson's regression model. Correlation analysis was performed using Spearman's test to assess the monotonic association between the monthly antimicrobial and PPIs consumption with CDI incidence rate. This non-parametric test was chosen due to its robustness to non-normal distributions and potential non-linear relationships. The incidence of CDI and the antimicrobial and PPI consumption were paired without time intervals and with 1-month interval to determine the immediate and delayed effects of drug consumption on the incidence of CDI. The second stage of the analysis included interrupted time-series methods to evaluate the impact of key temporal breakpoints on CDI incidence rate. A segmented Poisson's regression model was employed, given its appropriateness for analyzing count data with time-dependent changes. The interrupted time-series allows for the evaluation of immediate changes in level, coupled with long-term effects due to trend changes. The time series is divided (interrupted) into pre and post breakpoints. Least square regression lines are fitted to each segment of the time series and together expressed by the following model:

$$Yt = \beta 0 + \beta 1 * time1 + \beta 2 * breakepoint1 + \beta 3 * timea\ f\ terbreakepoint1 + et$$

Yt represents any outcome at time point (t), which in this case is CDI incidence. Time is a continuous variable, measuring the number of months included in the entire time frame (60 months). β0 estimated the baseline level at time point zero (y-axis intercept), β1 estimates the baseline trend, β2 estimates the level change between the pre and post breakpoint period, and β3 estimates the change in trend after the breakpoint. The sum of β1 and β3 estimates the post-breakpoint slope. The error et represents the variability of the model. We distinguished three main breakpoints. Firstly, one year after the implementation of the ASP in the participating hospitals (January 2019). This was followed by the second and third periods, obtained during the COVID-19 pandemic, the first of which is set in March 2020, the month when the COVID-19 pandemic was officially declared; followed by September 2020, which is the month with the lowest rate of nosocomial CDI in the included time series. p values < 0.05 were considered statistically significant. Statistical analysis was performed using SAS Studio OnDemand for Academics (SAS Institute Inc., Cary, NC, USA) Version: 3.81 (Edition Enterprise).

4

Results

This thesis per compilation of publications, includes two articles about antimicrobial and PPI consumption trends and their association with nosocomial CDI at ecological level. The articles presented and attached to this thesis were accepted by the Academic Committee of the Doctoral Program in Pharmacology. Moreover, an individual results analysis of the population-level study is provided below.

4.1 Antimicrobial, PPIs exposure and risk factors for hospital-acquired *Clostridiodes difficile* infection: A propensity score matched study

During the study period a total of 354 cases of HA-CDI were identified, the lowest rate of infection was found in September 2020 with 0.009 CDI per 100 bed days and the highest rate in February 2020 with 0.071 CDI per 100 bed days. On the other hand, 1172 controls were recognized. Clinical and demographic data stratified by the presence or absence of CDI before and after propensity score analysis are show in table 3.

Table 4 Baseline and clinical characteristics of patients by *Clostridiodes difficile* infection group

Patient characteristics	Whole cohort (n=1526)			Propensity score matched cohort (n=568)		
	CDI (n=354)	Control (n=1172)	p Value	CDI (n=284)	Control (n=284)	p Value
Demographics						
Age, years (mean)	55.75	57.31	0.82	55.90	56.81	0.26
Male gender	167 (47.18)	559 (47.70)	0.59	132 (46.48)	131 (46.13)	1.00
Hospitalization ward						
Intensive Care Unit	14 (3.95)	86 (7.42)	0.99	11 (3.87)	23 (8.10)	0.05
No intensive Care Unit	340 (96.05)	1085 (92.58)	0.99	273 (96.13)	261 (91.90)	0.05
Medical-surgical ward admission within 8 weeks	131 (37.01)	117 (9.98)	**<.0001**	73 (25.70)	73 (25.70)	1.00
Underlying diseases						
Comorbidities	330 (93.22)	1123 (95.82)	0.98	262 (92.25)	273 (96.13)	0.07
No of comorbidities (mean)	2.86	2.91	**<.0001**	2.76	2.97	0.37
Charlson Comorbidity Index (mean)	3.75	3.69	0.95	3.57	3.55	0.84
Congestive heart failure	53 (14.97)	183 (15.61)	0.64	46 (16.20)	41 (14.44)	0.64
Peripheral Vascular Disease	29 (8.19)	100 (8.53)	0.61	20 (7.04)	17 (5.99)	0.73
Myocardial infarction	10 (2.82)	34 (2.90)	0.58	10 (3.52)	4 (1.41)	0.17
Cerebrovascular disease	4 (1.13)	35 (2.99)	0.98	4 (1.41)	9 (3.17)	0.26
Cognitive impairment	5 (1.41)	26 (2.22)	0.87	4 (1.14)	4 (1.14)	1.00
Chronic obstructive pulmonary disease	7 (1.98)	33 (2.82)	0.85	7 (2.46)	7 (2.46)	1.00
Connective tissue disease	21 (5.93)	39 (3.33)	**0.02**	10 (3.52)	14 (4.93)	0.53
Peptic ulcer disease	12 (3.39)	51 (4.35)	0.82	8 (2.82)	10 (3.52)	0.81

Patient characteristics	Whole cohort (n=1526)			Propensity score matched cohort (n=568)		
	CDI (n=354)	Control (n=1172)	p Value	CDI (n=284)	Control (n=284)	p Value
Mild hepatic disease	3 (0.85)	34 (2.90)	0.99	3 (1.06)	6 (2.11)	0.50
Severe hepatic disease	19 (5.37)	55 (4.69)	0.34	17 (5.99)	13 (4.58)	0.57
Diabetes Mellitus without complications	114 (32.20)	341 (29.10)	0.15	90 (31.69)	81 (28.52)	0.46
Diabetes Mellitus with complications	21 (5.93)	55 (4.69)	0.21	16 (5.63)	16 (5.63)	1.00
Hemiplegia or paraplegia	8 (2.26)	11 (0.94)	0.05	6 (2.11)	4 (1.41)	0.75
Chronic kidney disease	82 (23.16)	208 (17.75)	**0.015**	65 (22.89)	53 (18.66)	0.25
History of malignancy	46 (12.99)	201 (17.15)	0.97	34 (11.97)	48 (16.90)	0.12
Metastatic cancer	5 (1.41)	11 (0.94)	0.30	0 (0.00)	2 (0.70)	0.50
Leukemia	21 (5.93)	56 (4.78)	0.22	14 (4.93)	23 (8.10)	0.17
Lymphoma	16 (4.52)	35 (2.99)	0.11	6 (2.11)	5 (1.76)	1.00
Human immunodeficiency virus	12 (3.39)	30 (2.56)	0.25	7 (2.46)	4 (1.41)	0.54
Septic shock	45 (12.71)	145 (12.37)	0.46	36 (12.68)	36 (12.68)	1.00
COVID-19	32 (9.04)	158 (13.48)	0.99	29 (10.21)	35 (12.32)	0.50
Use of immunosuppressive agents	70 (19.77)	122 (10.41)	**<.0001**	40 (14.08)	40 (14.08)	1.00
Concomitant infectious disease	219 (61.86)	656 (55.97)	**0.02**	180 (63.38)	180 (63.38)	1.00
Inflammatory bowel disease	9 (2.54)	26 (2.22)	0.42	9 (3.17)	9 (3.17)	1.00

In the unmatched cohort (n = 1526), patients with CDI (n = 354) differed significantly from controls (n = 1172) in several variables. Notably, CDI patients had a higher frequency of medical-surgical ward admission within the previous 8 weeks (37.0% vs. 10.0%, $p < 0.0001$), greater use of immunosuppressive agents (19.8% vs. 10.4%, $p < 0.0001$), higher prevalence of chronic kidney disease (23.2% vs. 17.8%, $p = 0.015$), and a greater number of comorbidities on average (2.86 vs. 2.91, $p < 0.0001$). Additionally, CDI cases had a higher prevalence of connective tissue disease (5.9% vs. 3.3%, $p = 0.02$) and concomitant infectious diseases (61.9% vs. 56.0%, $p = 0.02$).

After 1:1 PSM, 284 CDI patients were matched with 284 controls, resulting in improved balance across most covariates. There were no significant differences between matched groups in terms of age (55.90 vs. 56.81 years, $p = 0.26$), sex (46.5% vs. 46.1% male, $p = 1.00$), or Charlson Comorbidity Index (3.57 vs. 3.55, $p = 0.84$). The number of comorbidities also became comparable between groups (2.76 vs. 2.97, $p = 0.37$). Notably, previous medical-surgical ward admission within 8 weeks, immunosuppressive therapy use, and the presence of concomitant infectious diseases, all of which were significantly different in the unmatched cohort, became well balanced post-matching ($p = 1.00$ for each).

Although the overall distribution of baseline characteristics was well matched, a borderline significant difference was observed in the proportion of patients admitted to the intensive care unit (3.9% vs. 8.1%, $p = 0.05$), which may reflect residual imbalance.

A descriptive analysis of the characteristics of the complete case control is shown in table 4. The mean age of affected patients was 55.8 years, and 47.2% of cases occurred in males. Most episodes were recorded in medical-surgical wards (96.1%), with only 3.9% occurring in intensive care units. The average length of hospital stay was 33.8 days.

Regarding infection classification, 96.6% of episodes were new hospital-onset cases. In terms of severity, 65.5% of CDI episodes were classified as mild, 27.4% as severe, and 7.1% as severe-complicated. A total of 97.2% of patients received specific treatment for CDI. The most frequently used regimen was oral vancomycin combined with intravenous metronidazole (57.6%), followed by oral vancomycin in monotherapy (35.0%). A small proportion of patients (2.8%) received fecal microbiota transplantation (FMT). Among clinical outcomes, FMT failure was observed in 0.8% of patients. In-hospital mortality occurred in 24.6% of cases, and 16.7% of deaths were directly attributed to CDI.

The adjusted odds of CDI by individual antimicrobial and per groups after PSM is show in table 5. The use of multiple antimicrobials, before (at least in the last 8 weeks prior to hospital admission) and during hospital stay was associated with risk for developing nosocomial CDI (OR: 0.82 CI95% 0.73-0.92). Furthermore, a risk of CDI was observed with Anidulafungin-Caspofungin (OR: 011 CI95% 0.013-1.31, p=0.02), Cefalotin (OR: 0.51 CI95% 0.28-0.91, p=0.03) and Cefotaxime (OR: 1.61 CI95% 1.15-2.25, p=0.006) use. No statistically significant associations were found for carbapenems, fluroquinolones or clindamycin agents classified in the literature as high-risk antibiotics. El use of PPIs neither demonstrated a positive association as a risk factor for nosocomial CDI in our hospitals.

Table 5. Characteristics of Hospital Acquired *Clostridioides difficile* infection (HA-CDI)

Characteristics	Whole cohort (n=354)
Total of hospital onset episodes	354
Age (mean)	55.75
Male	167 (47.18)
Current hospital ward	
Intensive Care Unit	14 (3.95)
Medical-Surgical	340 (96.05)
Lenght of stay (mean)	33.78
Hospital associated infection classification	
New hospital onset	342 (96.61)
Hospital first recurrence	8 (2.26)
Hospital second recurrence	2 (0.56)
CDI episode severity	
Mild	232 (65.54)
Severe	97 (27.40)
Severe complicated	25 (7.06)
Received CDI treatment	
No treatment	10 (2.82)
Oral Metronidazole	16 (4.52)
Oral Vancomycin	124 (35.03)
Oral Vancomycin + IV Metronidazole	204 (57.63)
Fecal microbiota transplantation	10 (2.82)
Clinical outcomes	
Fecal microbiota transplantation failure	3 (0.84)
In hospital death	87 (24.58)
CDI related death	59 (16.67)

Data are reported as the no (%), unless otherwise noted

Table 6 Adjusted risk of HA-CDI with antimicrobials and proton pump inhibitors

Antimicrobial	Propensity score matched cohort (n=568)		Odds ratio (95 % IC)	p value
	CDI (n=284)	Control (n=284)		
Antibiotics	261 (91.90)	252 (88.73)	1.44 (0.82-2.53)	0.25
Number of antibiotics used (mean)	2.53	2.70	0.82 (0.73-0.92)	**0.0011**
Antivirals	2 (0.70)	3 (1.06)	0.66 (0.11-4.00)	1.00
Acyclovir	1 (0.35)	3 (1.06)	0.33 (0.034-3.20)	0.62
Valganciclovir	1 (0.35)	0 (0.0)	0.49 (0.45-0.54)	1.00
Antifungals	16 (5.63)	23 (8.10)	0.67 (0.35-1.31)	0.31
Anidulafungin-Caspofungin	1 (0.35)	9 (3.17)	0.11 (0.013-0.85)	**0.02**
Fluconazole	15 (5.28)	15 (5.28)	1.00 (0.47-2.08)	1.00
Itraconazole	1 (0.35)	0 (0.0)	0.49 (0.45-0.54)	1.00
Aminoglycosides	41 (14.44)	40 (14.08)	1.02 (0.64-1.64)	1.00

Antimicrobial	Propensity score matched cohort (n=568)		Odds ratio (95 % IC)	p value
	CDI (n=284)	Control (n=284)		
Amikacin	23 (8.10)	26 (9.15)	0.87 (0.48-1.57)	0.76
Gentamicin	18 (6.34)	14 (4.93)	1.30 (0.63-2.67)	0.58
Monobactams y Carbapenems	75 (26.41)	67 (23.59)	1.16 (0.79-1.70)	0.49
Aztreonam	2 (0.70)	2 (0.70)	1.00 (0.14-7.15)	1.00
Ertapenem	22 (7.75)	24 (8.45)	0.90 (0.49-1.66)	0.87
Imipenem-cilastatine	1 (0.35)	0 (0.0)	0.49 (0.49-0.54)	1.00
Meropenem	59 (20.77)	52 (18.31)	1.16 (0.77-1.77)	0.52
Penicillins	67 (23.59)	70 (24.65)	0.94 (0.64-1.38)	0.84
Amoxicillin	8 (2.82)	16 (5.63)	0.48 (0.20-1.15)	0.14
Ampicillin	29 (10.21)	25 (8.80)	1.17 (0.67-2.06)	0.67
Ampicillin/sulbactam	1 (0.35)	1 (0.35)	1.00 (0.062-16.06)	1.00
Sodium penicillin	3 (1.06)	1 (0.35)	3.02 (0.31-29.22)	0.62
Oxacillin	12 (4.23)	13 (4.58)	0.91 (0.41-2.05)	1.00
Piperacilllin/tazobactam	26 (9.15)	25 (8.80)	1.04 (0.58-1.85)	1.00
Cephalosporins	229 (80.63)	212 (74.65)	1.41 (0.95-2.10)	0.10
Cephalexin	9 (3.17)	8 (2.82)	1.13 (0.42-2.96)	1.00
Cefalotina	19 (6.69)	35 (12.32)	0.51 (0.28-0.91)	**0.03**
Cefotaxime	180 (63.38)	147 (51.76)	1.61 (1.15-2.25)	**0.006**
Ceftaroline	2 (0.70)	3 (1.06)	0.66 (0.11-4.00)	1.00
Ceftazidime	59 (20.77)	56 (19.72)	1.06 (0.70-1.60)	0.83
Ceftazidime/avibactam	2 (0.70)	3 (1.06)	0.66 (0.11-4.00)	1.00
Ceftolozane/tazobactam	0 (0.00)	2 (0.70)	0.49 (0.45-0.54)	0.49
Fluroquinolones	22 (7.75)	31 (10.92)	0.68 (0.38-1.21)	0.24
Ciprofloxacin	15 (5.28)	25 (8.80)	0.58 (0.29-1.12)	0.13
Levofloxacin	7 (2.46)	7 (2.46)	1.00 (0.34-2.88)	1.00
Macrolides	8 (2.82)	14 (4.93)	0.55 (0.23-1.35)	0.27
Azithromycin	1 (0.35)	1 (0.35)	1.00 (0.062-16.06)	1.00
Clarithromycin	7 (2.46)	13 (4.58)	0.52 (0.20-1.34)	0.25
Tetracyclines	4 (1.14)	10 (3.52)	0.39 (0.12-1.26)	0.17
Doxycycline	4 (1.41)	7 (2.46)	0.56 (0.16-1.95)	0.54
Tigecycline	0 (0.00)	3 (1.06)	0.49 (0.45-0.54)	0.25
Clindamycin	25 (8.80)	15 (5.28)	1.73 (0.89-3.35)	0.13
Linezolid	15 (5.28)	15 (5.28)	1.00 (0.47-2.08)	1.00
Metronidazole	36 (12.68)	53 (18.66)	0.63 (0.39-1.00)	0.06
Nitrofurantoin	11 (3.87)	14 (4.93)	0.77 (0.34-1.74)	0.68
Polymyxin B	0 (0.00)	2 (0.70)	0.49 (0.45-0.54)	0.49
Rifampicin	1 (0.35)	0 (0.00)	0.49 (0.45-0.54)	1.00
Trimethoprim/Sulfamethoxazole	39 (13.73)	53 (18.66)	0.69 (0.44-1.08)	0.13
Vancomycin	65 (22.89)	81 (28.52)	0.74 (0.51-1.08)	0.15
Proton Pump Inhibitors	145 (51.06)	147 (51.76)	0.97 (0.70-1.35)	0.93
Oral Proton Pump Inhibitors	110 (38.73)	122 (42.96)	0.83 (0.60-1.17)	0.34
Intravenous Proton Pump Inhibitors	19 (6.69)	10 (3.52)	0.75 (0.56-0.99)	0.12

Length of stay, the number of comorbidities and ICU stay were significantly associated with CDI in the univariable and multivariable model (see table 6 and 7 for complete description for possible clinical risk factors related with HA-CDI).

Table 7 Univariable logistic regression for factors associated with HA-CDI

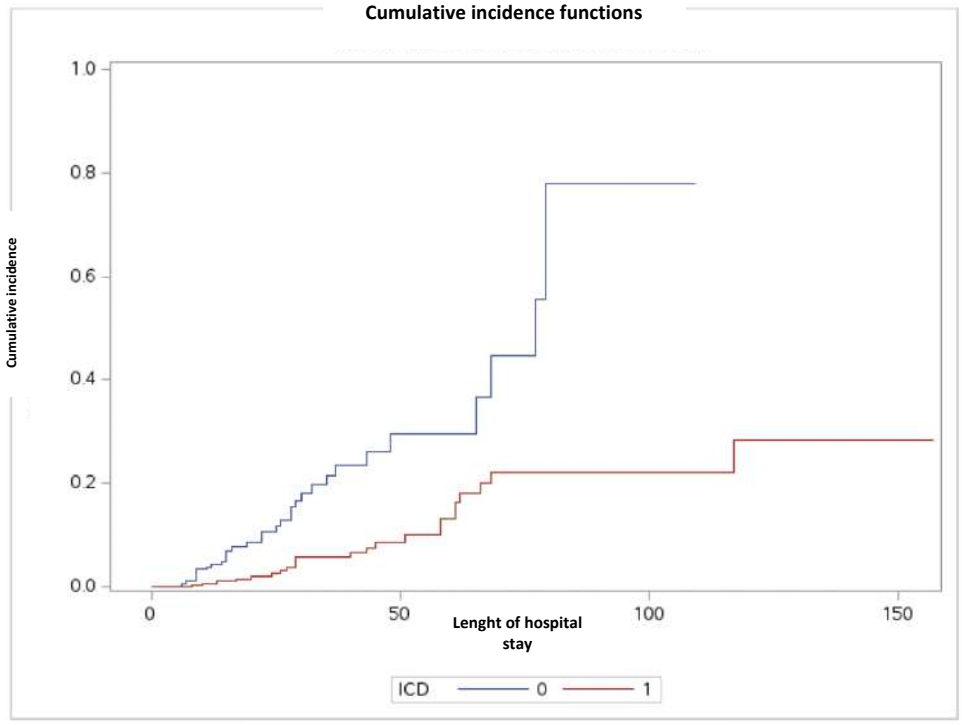
Characteristics	Propensity score matched cohort (n=568)		Odds ratio (95 % IC)	p Value
	CDI (n=284)	Control (n=284)		
Demographics				
Age, years (mean)	55.90	56.81	0.99 (0.98-1.00)	0.41
Male gender	132 (46.48)	131 (46.13)	1.01 (0.72-1.41)	1.00
Hospitalization ward				
Intensive Care Unit	11 (3.87)	23 (8.10)	0.45 (0.21-0.95)	0.05
No intensive Care Unit	273 (96.13)	261 (91.90)	0.45 (0.22-0.95)	0.05
Length of stay (mean)	33.19	20.39	1.04 (1.02-1.05)	**<.0011**
Hospital ward admission within 8 weeks	73 (25.70)	73 (25.70)	1.00 (0.68-1.45)	1.00
Underlying diseases				
Comorbidities	262 (92.25)	273 (96.13)	0.47 (0.22-1.00)	0.07
No of comorbidities (mean)	2.76	2.97	0.84 (0.72-0.99)	**0.04**
Charlson Comorbidity Index (mean)	3.57	3.55	1.08 (0.95-1.24)	0.21
Congestive heart failure	46 (16.20)	41 (14.44)	1.14 (0.72-1.80)	0.64
Peripheral Vascular Disease	20 (7.04)	17 (5.99)	1.18 (0.60-2.32)	0.73
Myocardial infarction	10 (3.52)	4 (1.41)	2.55 (0.79-8.24)	0.17
Cerebrovascular disease	4 (1.41)	9 (3.17)	0.43 (0.13-1.43)	0.26
Cognitive impairment	4 (1.41)	4 (1.41)	1.00 (0.24-4.03)	1.00
Chronic obstructive pulmonary disease	7 (2.46)	7 (2.46)	1.00 (0.34-2.88)	1.00
Connective tissue disease	10 (3.52)	14 (4.93)	0.70 (0.30-1.61)	0.53
Peptic ulcer disease	8 (2.82)	10 (3.52)	0.79 (0.30-2.04)	0.81
Mild hepatic disease	3 (1.06)	6 (2.11)	0.49 (0.12-1.99)	0.50
Severe hepatic disease	17 (5.99)	13 (4.58)	1.32 (0.63-2.78)	0.57
Diabetes Mellitus without complications	90 (31.69)	81 (28.52)	1.16 (0.81-1.66)	0.46
Diabetes Mellitus with complications	16 (5.63)	16 (5.63)	1.00 (0.49-2.04)	1.00
Hemiplegia or paraplegia	6 (2.11)	4 (1.41)	1.51 (0.42-5.41)	0.75
Chronic kidney disease	65 (22.89)	53 (18.66)	1.29 (0.86-1.94)	0.25
History of malignancy	34 (11.97)	48 (16.90)	0.66 (0.41-1.07)	0.12
Metastatic cancer	2 (0.70)	0 (0.0)	0.49 (0.45-0.54)	0.49
Leukemia	14 (4.93)	23 (8.10)	0.58 (0.29-1.16)	0.17
Lymphoma	6 (2.11)	5 (1.76)	1.20 (0.36.3.99)	1.00
Human immunodeficiency virus	7 (2.46)	4 (1.41)	1.76 (0.51-6.11)	0.54
Septic shock	36 (12.68)	36 (12.68)	1.00 (0.61-1.63)	1.00
COVID-19	29 (10.21)	35 (12.32)	0.80 (0.48-1.36)	0.50
Use of immunosuppressive agents	40 (14.08)	40 (14.08)	1.00 (0.62-1.60)	1.00
Concomitant infectious disease	180 (63.38)	180 (63.38)	1.00 (0.71-1.40)	1.00

Characteristics	Propensity score matched cohort (n=568)		Odds ratio (95 % IC)	p Value
	CDI (n=284)	Control (n=284)		
Inflammatory bowel disease	9 (3.17)	9 (3.17)	1.00 (0.39-2.55)	1.00

Table 8 Multivariable logistic regression for factors associated with HA-CDI

Variable	Odds ratio (95% IC)	p Value
Intensive care unit (current admission)	4.14 (1.79-0.97)	**0.0009**
Number of comorbidities	0.88 (0.78-0.98)	**0.02**
Length of stay	1.03 (1.02-1.05)	**<.0001**

Finally, an all-cause and disease-specific mortality analysis in hospitalized patients with *Clostridiodes difficile* infection was performed in the matched cohort of patients diagnosed with CDI. The Kaplan-Meir graphic (Figure 15) shows that in this cohort over time, the cumulative probability of dying from other causes is greater than the probability of death from CDI. This suggests that clinical management should adopt a comprehensive approach, addressing not only infection control but also the associated comorbidities.



Gray´s test for equality of cumulative incidence functions

Chi-square	DF	Pr>Chi-square
21.8261	1	<.0001

Figure 15. Kaplan-Meier Survival Curve for patients with HA-CDI over time to death
Blue line represents CDI related mortality, red line represents all-cause mortality

1.6 First study

"Trends in Antimicrobial Consumption in Tertiary Care Hospitals in Costa Rica from 2017 to 2021: A Comparative Analysis of Defined Daily Doses per 100 Bed Days and per 100 Discharges"

Fernández-Barrantes, C.; Ramos-Esquivel, A.; Hernández-Soto, L.E.; Ramírez-Cardoce, M.; Garro-Zamora, L.D.; Cordero, J.C.; Grau, S. Trends in Antimicrobial Consumption in Tertiary Care Hospitals in Costa Rica from 2017 to 2021: A Comparative Analysis of Defined Daily Doses per 100 Bed Days and per 100 Discharges. *Antibiotics* **2024**, *13*, 939. https://doi.org/10.3390/antibiotics13100939

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Trends in Antimicrobial Consumption in Tertiary Care Hospitals in Costa Rica from 2017 to 2021: A Comparative Analysis of Defined Daily Doses per 100 Bed Days and per 100 Discharges

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Abstract: Background: Antimicrobial consumption (AMC) data in Latin America are scarce and usually spread out across different sources used to make AMC calculations, making it difficult to both standardize and compare regions through similar time frames. The main objective was to analyze AMC trends in Social Security tertiary care hospitals in Costa Rica in the period spanning January 2017 to December 2021, using both the defined daily dose (DDD)/100 bed days and DDD/100 discharges. **Methods**: This is a retrospective observational study of antimicrobial consumption. Global consumption trends were calculated and expressed as DDD/100 bed days and DDD/100 discharges. Trends in antimicrobial consumption were analyzed using a simple linear regression model to determine potential differences in antimicrobial usage throughout the study's duration. **Results**: A statistically significant increase in the consumption expressed in DDD/100 discharges was observed in the following groups: carbapenems, 7.6% (trend: 64.68, $p < 0.0001$), trimethoprim-sulfamethoxazole: 12.6% (trend: 16.45, $p < 0.0001$), quinolones 9.4% (trend: 36.80, $p = 0.02$), vancomycin 2.0% (trend: 16.30, $p = 0.03$), echinocandins: 6.0% (trend: 15.17, $p = 0.01$) and azole antifungals: 12.10% (trend: 102.05, $p < 0.0001$). Additionally, a statistically significant increase of 10.30% in the consumption of azole antifungals expressed in DDD/100 bed days was observed ($p = 0.0008$). In contrast, a statistically significant decrease in consumption, expressed in DDD/100 discharges, was identified for cephalosporins −6.0% ($p < 0.0001$) and macrolides −16.5% ($p < 0.0001$). Macrolides also showed a downward trend in consumption, as expressed in DDD/100 bed days (−14.3%, $p < 0.0001$). According to World Health Organization (WHO) access, watch and reserve (AWaRe) classification trend analysis, only the reserve group showed a statistically significant upward change of 9.2% ($p = 0.016$). **Conclusions**: This five-year analysis demonstrated trends over time in overall antimicrobial consumption measured in DDD/100 bed days and DDD/100 discharge rates that correlate. In general, for all antimicrobials, after the implementation of antimicrobial stewardship programs (ASP), a downward trend is reported; in contrast, during the COVID-19 pandemic the AMC shows a general upward trend. The comparison between DDD/100 bed days and DDD/100 discharges allows for complementary comparisons to be made regarding antimicrobial exposure in a clinical setting.

Keywords: antimicrobial consumption; antimicrobial stewardship; defined daily dose

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1. Introduction

Antimicrobial resistance (AMR) poses a major threat to global human health [1]. A study published in 2019 established a link between AMR and approximately 4.95 million deaths, with increased projections to 10 million deaths per annuum by 2050 [2]. Liberal antimicrobial use is one of the main drivers of AMR; hence, the surveillance and optimal use of the latter are among the key strategies use to mitigate AMR and are included in the five main objectives of the World Health Organization (WHO)'s Global Action Plan (GAP) for AMR [3].

Antimicrobial usage primarily takes place in outpatient and community care. Nevertheless, in hospital environments, the intensity of antimicrobial consumption is significantly greater as vulnerable patients situated closely together, which increases the likelihood of the emergence and spread of resistant pathogens [4].

Additionally, fungal diseases represent a growing concern, with a significant impact on morbidity and mortality, as has been previously observed with bacteria; it is known that the improper use of antifungals poses a selective pressure that is a key driver for the emergence and dissemination of antifungal-resistant strains in clinical settings [5,6].

Monitoring antimicrobial consumption (AMC) in health facilities is an important element of all antimicrobial stewardship programs (ASP) [7]. In facilitating the standardizing of results and the ability to compare consumption information across time and different hospitals, the most widely implemented system is the defined daily dose (DDD), according to the WHO Anatomical Therapeutic Chemical (ATC) system [8–10]. Standardizing measures of hospital activity and hospital consumption data must correspond strictly to the time people are under surveillance and the hospital activity units covered (bed days and/or discharges) [4,9]. The purpose is to identify areas in need of improvement and assess interventions by comparing hospitals of the same complexity and by evaluating time series data from a single center, while providing support for studying ecological effects [8,11].

Another antimicrobial stewardship (AMS) tool used to monitor AMC is the WHO access, watch and reserve (AWaRe) classification of antibiotics, developed in 2017. This tool classifies antibiotics into three groups, taking into account the impact of different antibiotics on AMR, to emphasize the importance of their appropriate use [4]. The access group includes first or second choices for the treatment of bacterial infections while minimizing the potential of AMR. Antibiotics in the watch group are recommended as a first or second choice for the empiric treatment of a specific, limited number of infective syndromes, but have, in general, a strong potential for generating AMR in bacteria [11]. The reserve group includes antibiotics and antibiotic classes that should be reserved for the treatment of confirmed or suspected infections due to multidrug-resistant (MDR) organisms; in many scenarios these agents are the only therapeutic option [4].

In Costa Rica, a country with a total population of 5,044,197 inhabitants, public healthcare facilities belong to the Social Security, Caja Costarricense de Seguro Social (CCSS), network model, which is independent from the Ministry of Health. This model is based on three attention levels, where the third level stands as the one of maximum complexity. Furthermore, an institutional medicines policy has been developed based on the WHO List of Essential Medicines model, which seeks to satisfy most of the population's health necessities. Medication is accessible to patients through the universal coverage provided by CCSS [12–15].

Nonetheless, the Ministry of Health of Costa Rica established its own National Action Plan to Mitigate Antimicrobial Resistance in 2018, under which the surveillance of AMC in healthcare settings is mandatory. This initiative is yet to be implemented, thus rendering knowledge on this topic scarce across Costa Rica and its neighboring Central American nations [16,17].

Antimicrobial stewardship programs have been implemented in different healthcare hospitals in Costa Rica during the past decade; one example is Clinica Biblica Hospital, a private institution with 68 beds in San José, Costa Rica, where, in 2015, an AMS was implemented. The program has been continuously monitored by a multidisciplinary team,

consisting of a clinical pharmacist (director), an infectious disease expert, a microbiologist and the hospital's assistant chief medical officer. In addition, since 2018, AMSs have been implemented in Social Security tertiary care hospitals following a multidisciplinary approach; the main objectives have focused on educational strategies, the application and tracking of antimicrobial guidelines, diagnostic stewardship, monitoring AMC and evaluating economic outcomes [18–20].

The objective of the present study was to describe and analyze antimicrobial consumption trends in Social Security's tertiary care hospitals across Costa Rica and throughout the period of 2017–2021, using both DDD/100 bed days and DDD/100 discharges as measurements of hospital activity.

2. Results

2.1. Indicators of Hospital Activity

Table 1 presents the mean of unadjusted DDDs, bed days, discharges and lengths of stay. During these five years, a slight increase in DDD was observed. In contrast, the number of bed days and discharges showed a downward trend (−0.13% and −0.15%, respectively). Contrastingly, the length of reported stays did not demonstrate a statistically significant change in the same time frame.

Table 1. Mean of defined daily doses (DDDs), bed days, discharges and lengths of stay in 2017 and 2021 among tertiary care hospitals in Costa Rica.

Mean	Year		Variation (%)	*p* Value
	2017	2021		
DDDs	**622.94**	890.74	0.43	**0.0004**
Bed days	14,478.46	12,555.54	−0.13	**0.0010**
Discharges	2011.75	1702.83	−0.15	**0.0004**
Length of stay	7.33	7.48	0.02	0.06

DDD: defined daily dose (unadjusted); %: difference between 2017 and 2021; statistically significant *p* values are in bold.

2.2. Antimicrobial Consumption Patterns and Trends in the Period 2017–2021

Table 2 shows a description of the AMC patterns seen during each year of this study period; the top ten most common antimicrobials are reported in %DDD/100 bed days and %DDD/100 discharges. Figure 1 gives a graphical report of the top 10 most common antimicrobials used using both quantification indicators. According to our results, the top three most consumed antimicrobials are Cefotaxime, at 13% of the total of the AMC in both DDD/100 bed days and DDD/100 discharges; Vancomycin, at approximately 10% in both indicators; and Oxacillin, at 8.5% DDD/100 bed days and 8.1% DDD/100 discharges.

Table 2. Top 10 ranking of antimicrobial consumption, expressed in DDD/100 bed days and DDD/100 discharges.

DDD/100 Bed Days						DDD/100 Discharges					
Antimicrobials	2017	2018	2019	2020	2021	Antimicrobials	2017	2018	2019	2020	2021
Amikacin	53.68	41.5	52.41	77.95	61.6	Amikacin	239.96	183.35	231.96	223.16	363.19
Benzylpenicillin	54.12	42.16	34.97	38.9	27.93	Benzylpenicillin	322.02	264.01	222.38	182.34	176.69
Cefotaxime	**223.02**	**217.85**	**210.53**	**275.23**	**167.82**	Cefotaxime	**1215.25**	**1103.98**	**1152.61**	**1128.61**	**111.03**
Ceftazidime	80.99	64.99	62.13	108.61	70.3	Ceftazidime	435.95	322.11	354.17	414.51	473.03
Fluconazole	103.82	101.22	101.73	147.86	**147.18**	Fluconazole	563.25	**562.67**	**668.1**	696.02	**942.6**
Meropenem	130.24	**120.78**	**137.42**	**190.36**	102.55	Meropenem	613.8	509.41	639.28	601.58	602.72
Metronidazole	63.09	60.33	47.58	52.32	36.00	Metronidazole	359.01	316.41	293.89	259.75	231.69

Table 2. *Cont.*

Antimicrobials	DDD/100 Bed Days 2017	2018	2019	2020	2021	Antimicrobials	DDD/100 Discharges 2017	2018	2019	2020	2021
Oxacillin	**145.52**	114.92	117.1	178.03	**149.14**	Oxacillin	**718.02**	557.74	579.35	627.01	**882.98**
Sulfamethoxazole/ Trimethoprim	74.42	77.82	100.6	176.83	107.19	Sulfamethoxazole/ Trimethoprim	475.2	515.55	619.71	**780.73**	772.85
Vancomycin	**176.02**	**166.40**	**181.72**	**241.48**	**139.66**	Vancomycin	**814.88**	**773.91**	**820.56**	**809.67**	878.52

DDD: defined daily dose; the top three antimicrobials per year are in bold.

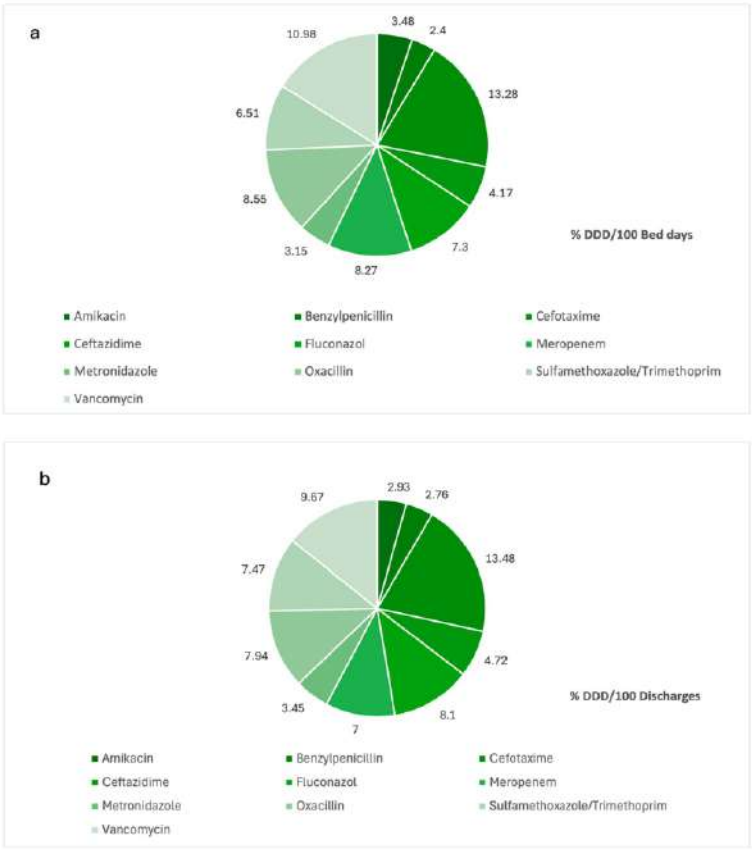

Figure 1. Consumption of antimicrobials ranked in top 10, expressed in % of DDD/bed days (**a**) and % DDD/100 discharges (**b**), from 2017 to 2021 in tertiary care hospitals in Costa Rica. DDD: defined daily dose.

As a complement to the patterns shown previously, a complete description of the AMC distribution per antimicrobial group is shown in Figure 2.

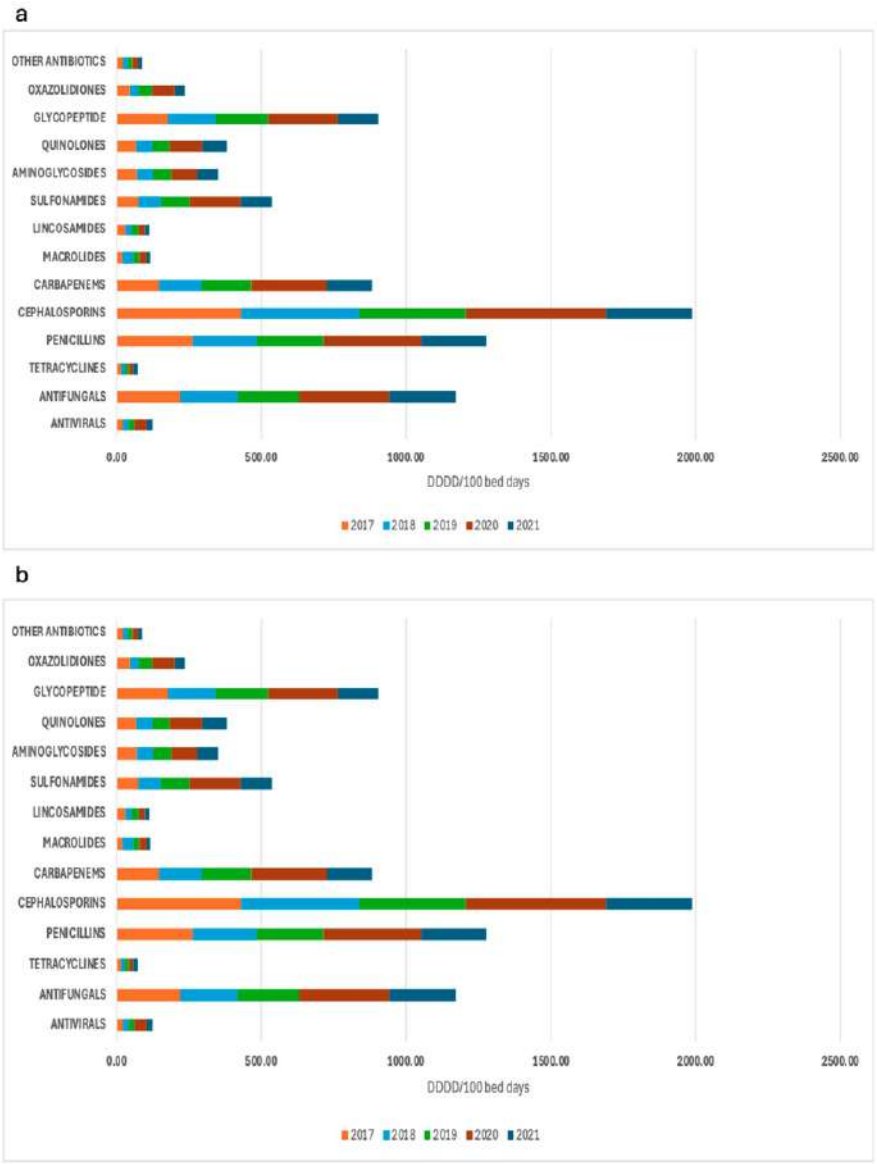

Figure 2. Total antimicrobial consumption per selected group, expressed in DDD/100 bed days (**a**) and DDD/100 discharges (**b**), from 2017 to 2021. DDD: defined daily dose.

Global trends in antimicrobial consumption during this period are shown in Table 3. Evidently, the overall consumption of antimicrobial medication increased, in terms of DDD/100 discharges, from 270.63 to 313.25 (+2.97%, $p = 0.0007$). On the other hand, the consumption calculated in DDD/100 bed days showed no statistically significant changes,

ranging from 61.35 to 61.78 (+0.14%, $p = 0.31$). In this general overview, despite antibiotics having a decreased use, they still showed a significantly increased use when compared to antivirals. However, no statistical difference was discerned across the period (Figure 3). Antifungals were the only group with a statistically significant increase in DDD/100 discharges: from 80.41 to 123.52 (+8.97%, $p < 0.0001$).

Table 3. Antimicrobial consumption (J01, J02, J05) in tertiary care hospitals in Costa Rica from 2017 to 2021, expressed in DDD/100 bed days and DDD/100 discharges.

Total	2017	2018	2019	2020	2021	% AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
Total antimicrobials (J01, J02, J05)									
DDD/100 bed days	61.35	54.30	60.13	93.05	61.78	0.14	(−32.11 to 32.39)	3.96	0.31
DDD/100 discharges	270.63	245.68	277.80	295.44	313.25	2.97	(−5.25 to 11.19)	13.50	**0.0007**
Total antibiotics (J01)									
DDD/100 bed days	30.36	27.92	28.67	40.86	25.51	−3.42	(−32.78 to 25.94)	0.32	0.84
DDD/100 discharges	164.29	149.18	155.53	154.64	161.92	0.29	(−5.85 to 5.27)	0.072	0.96
Total antifungals (J02)									
DDD/100 bed days	26.30	22.68	27.60	43.21	32.49	4.31	(−28.22 to 36.84)	3.29	0.05
DDD/100 discharges	80.41	73.59	94.49	104.61	123.52	8.97	(−4.67 to 22.61)	11.72	**<0.0001**
Total antivirals (J05)									
DDD/100 bed days	4.69	3.70	3.87	8.98	3.77	−4.27	(−74.42 to 65.88)	−0.34	0.57
DDD/100 discharges	25.93	22.91	27.78	36.19	27.81	1.41	(−21.12 to 23.94)	1.70	0.14

DDD: defined daily dose; %AAPC: average annual percent change; 95% CI: 95% confidence intervals; Trend β1: the estimated change in antimicrobial consumption per year; statistically significant *p* values are in bold.

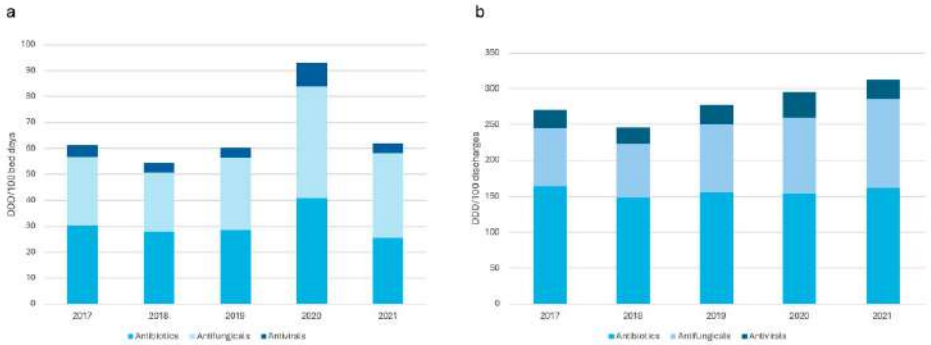

Figure 3. Total use of antimicrobials, expressed in DDD/100 bed days (**a**) and DDD/100 discharges (**b**), in tertiary care hospitals in Costa Rica during 2017–2021.

Detailed AMC trends for the main antimicrobial groups are shown in Table 4. The following groups showed a statistically significant increase in consumption, measured in DDD/100 discharges: carbapenems, from 699.00 (2017) to 948.00 (2021), representing an increase of 7.6% ($p < 0.0001$); trimethoprim-sulfamethoxazole, from 475.60 (2017) to 772.85 (2021), with an increase of 12.6% ($p < 0.0001$); quinolones 9.4% ($p = 0.02$); vancomycin, from 814.88 (2017) to 878.52 (2021), with an increase of 2.0% ($p = 0.03$); echinocandins, from 156.94 (2017) to 189.48 (2021), increasing their consumption by 6.0%; and azole antifungals, from 647.18 (2017) to 1045.72 (2021), with an increase of 12.1% ($p < 0.0001$). Also, a statistically significant increase of 10.30% in the consumption of azole antifungals, expressed in

DDD/100 bed days, was reported, from 116.52 (2017) to 163.64 (2021) ($p = 0.0008$). In contrast, a statistically significant decrease in consumption, expressed in DDD/100 discharges, was identified for cephalosporins—of −6.0%, from 2495.15 (2017) to 1946.72 (2021)—and macrolides—of −16.5%, from 360.60 (2017) to 174.26 (2021) ($p < 0.0001$). Macrolides also showed a downward trend in consumption, expressed in DDD/100 bed days, from 50.42 to 25.39 (−14.3%, $p = 0.005$). Figure 4 shows two stacked-line graphical descriptions of the monthly AMC trends for the most common antimicrobial groups used, expressed in DDD/100 bed days and DDD/100 discharges. The areas between the lines show the cumulative sum of all categories, presenting the total consumption of antimicrobials. After the implementation of ASP in our hospitals, a downward trend was reported, which is in contrast to during the first year of COVID-19 pandemic, where the AMC shows a general upward trend. According to our WHO AWaRe classification trend analysis, a statistically significant upward change of only 9.2% ($p = 0.016$) was shown. A complete description of the AWaRe classification trends is reported in Table 5.

Table 4. Antimicrobial consumption (J01, J02 and J05) trends for the main groups of antimicrobials from 2017 to 2021, expressed in DDD/100 bed days and DDD/100 discharges.

Total	2017	2018	2019	2020	2021	%AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
Tetracyclines (J01A)									
DDD/100 bed days	15.14	13.83	12.72	16.86	13.78	−0.8	−18.0 to 16.4	0.031	0.94
DDD/100 discharges	88.75	93.84	91.26	91.37	88.57	0.0	−3.1 to 3.1	−0.28	0.63
Penicillins (J01C)									
DDD/100 bed days	264.03	218.55	233.13	339.47	221.26	0.0	−26.4 to 26.4	3.53	0.80
DDD/100 discharges	1390.00	1165.89	1212.08	1278.22	1344.66	−0.4	−8.4 to 7.6	2.16	0.93
Cephalosporis (J01D)									
DDD/100 bed days	430.66	405.92	369.09	485.73	296.08	−5.8	−27.8 to 16.2	−18.93	0.29
DDD/100 discharges	2495.15	2313.39	2239.63	1999.53	1946.72	−6.0	−8.8 to −3.1	−141.07	**<0.0001**
Carbapenems, monobactams (J01DH, J01DF)									
DDD/100 bed days	146.13	147.72	172.54	260.09	156.77	6.6	−21.7 to 35.0	13.36	0.27
DDD/100 discharges	699.00	644.69	791.78	793.57	948.00	7.6	−2.7 to 17.9	64.68	**<0.0001**
Macrolides, lincosamides (J01F)									
DDD/100 bed days	50.42	61.99	44.17	46.65	25.39	−14.3	−40.0 to 11.4	−6.54	**0.005**
DDD/100 discharges	360.60	347.88	305.84	240.73	174.26	−16.5	**−24.4** to −8.7	−47.98	**<0.0001**
Sulfonamides and trimethoprim (J01E)									
DDD/100 bed days	74.42	77.82	100.60	176.83	107.19	15.9	−20.3 to 52.1	16.45	0.06
DDD/100 discharges	475.60	515.66	616.71	780.73	772.85	12.6	3.7 to 21.5	85.95	**<0.0001**
Aminoglycosides (J01G)									
DDD/100 bed days	69.62	56.59	63.76	88.93	71.01	3.0	−18.5 to 24.5	3.51	0.24
DDD/100 discharges	339.28	258.60	307.13	277.29	419.03	8.4	−16.6 to 33.3	17.81	0.26
Quinolones (J01M)									
DDD/100 bed days	68.01	56.02	59.38	113.04	84.09	13.2	−27.1 to 53.5	8.91	0.08
DDD/100 discharges	389.65	332.23	329.36	424.43	527.55	9.4	−6.3 to 25.1	36.80	**0.02**
Other antibacterials (J01XA01): Vancomycin									
DDD/100 bed days	176.02	166.40	181.72	241.48	139.66	−1.6	−25.4 to 22.2	0.23	0.98
DDD/100 discharges	814.88	773.91	820.56	809.67	878.52	2.0	−2.8 to 6.7	16.30	**0.03**

Table 4. *Cont.*

Total	2017	2018	2019	2020	2021	%AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
Other antibacterials (J01XX08): Linezolid									
DDD/100 bed days	46.41	28.18	47.76	76.56	34.61	1.8	−41.7 to 45.3	2.47	0.62
DDD/100 discharges	175.82	121.69	185.60	190.10	202.50	3.1	−17.1 to 23.4	12.17	0.08
Azole Antifungals (J02AB, J02AC)									
DDD/100 bed days	116.52	105.78	124.21	179.35	163.64	10.3	−8.9 to 29.6	16.78	**0.0008**
DDD/100 discharges	647.18	599.22	778.78	822.70	1045.72	12.1	0.0 to 24.2	102.05	**<0.0001**
Echinocandins Antifungals (J02AX)									
DDD/100 bed days	41.26	30.28	41.36	79.92	31.33	8.2	−42.9 to 59.2	2.97	0.59
DDD/100 discharges	156.94	136.72	166.16	223.39	189.48	6.0	−12.3 to 24.4	15.17	**0.01**
Antivirals (J05A)									
DDD/100 bed days	23.45	18.49	19.34	44.88	18.86	14.3	−48.3 to 77.0	1.72	0.57
DDD/100 discharges	25.93	22.91	27.78	38.19	27.81	4.0	−18.0 to 26.0	1.70	0.14

DDD: defined daily dose (unadjusted); % AAPC: average annual percent change, 95% CI: 95% confidence intervals; Trend β1: the estimated change in antimicrobial consumption per year; statistically significant *p* values are in bold.

Figure 4. Monthly antimicrobial consumption trends for selected groups, in DDD/100 bed days (**a**) and DDD/100 discharges (**b**), from 2017 to 2021. DDD: defined daily dose.

Table 5. Antibiotic consumption trends according to WHO AWaRe classification, expressed in DDD/100 bed days and DDD/100 discharges.

Total	2017	2018	2019	2020	2021	% AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
ACCESS									
DDD/100 bed days	621.18	553.66	539.78	748.08	519.41	−4.4	−47.6 to 45.0	−0.91	0.97
DDD/100 discharges	3452.78	3173.04	3161.55	3030.79	3209.69	−1.8	−22.2 to 7.8	−62.84	0.05
WATCH									
DDD/100 bed days	746.69	723.13	752.68	1079.66	623.95	−4.4	−55.3 to 56.4	11.1	0.81
DDD/100 discharges	3872.60	3550.61	3809.19	3992.44	3981.68	0.7	−9.6 to 11.3	55.99	0.14
RESERVE									
DDD/100 bed days	58.42	35.33	54.68	92.34	55.34	−1.3	−82.8 to 104.8	5.08	0.34
DDD/100 discharges	230.83	152.55	225.53	249.4	328.01	9.2	−42.3 to 70.3	29.12	**0.016**

WHO AWaRe: World Health Organization antibiotic classification (access, watch and reserve); DDD: defined daily dose; %AAPC: average annual percent change; 95% CI: 95% confidence intervals; Trend β1: the estimated change in antimicrobial consumption per year; statistically significant *p* values are in bold.

3. Discussion

Latin American countries do not regularly measure their antimicrobial consumption, and only a few occasionally review the overall consumption of antibiotics within their territory [17]. Due to these inconsistencies, data in this region are scarce and usually spread out over different sources used to make AMC calculations, making it difficult to both standardize and compare regions through similar time frames.

This is the first multicentric study with a standardized methodology that analyzes antimicrobial consumption trends in Social Security acute tertiary care hospitals in Costa Rica. Furthermore, another advantage this study presents is that it was carried out with data from the two hospitals in Costa Rica with the greatest complexity. Monitoring the consumption of antimicrobials in the hospital setting is a necessary measure, both for cost optimization reasons and to develop further strategies to suppress the appearance of resistant strains. Additionally, this study may become useful when evaluation stewardship programs follow results and trends that have been presented previously [9,21].

Variations in in-hospital antimicrobial use may be due to several factors. Firstly, it may depend on both hospital and patient characteristics, in addition to any pre-existing antibiotic policies, as well as the physician's education or the beliefs held by different healthcare systems. However, a substantial part of the differences in AMC metrics may be the result of differences in the methods used to measure antimicrobial use [22].

The DDD is the most accepted measure of AMC, and it refers to the average maintenance dose assumed for a drug´s main indication in adults [20]. The ATC/DDD system from the WHO allows for data collection at an aggregated level and avoids the need for individual-level data. This versality allows resource-limited countries to leverage existing data sources to develop sustainable AMC monitoring systems [17].

Even though the ATC/DDD system for all drugs has been available since the 1980s, it was not widely used, and in some cases it was misunderstood. This resulted in widespread confusion, due to misinformed publications, along with the incomplete and unspecified publication of antibiotic guidelines [22,23].

Most publications that quantify antimicrobial consumption trends in the hospital setting using this methodology use DDD/100 bed days as the standard denominator. This parameter reflects the hospital's exposure to antimicrobials, but does not describe either number or proportion of patients treated with antimicrobials, while parameters such as DDD/100 discharges provides information on the exposure per patient and helps to interpret consumption trends over time, as it shows changes in hospital activity [21,24].

The most common antimicrobials consumed (Figure 1 and Table 2) in our tertiary care hospitals from 2017 to 2021 were cefotaxime, vancomycin, oxacillin and fluconazole. The high proportion of cefotaxime and third-generation cephalosporins in general may be explained by different reasons; for example, due to its broad-spectrum activity, cefotaxime is prescribed as an empirical treatment option, and also it is believed that cephalosporins tend to be less allergenic compared to penicillins [25].

Recently, prescription numbers for cephalosporins from a national healthcare database from the United States reported that 47.5% of all antibiotics used corresponded this antimicrobial family, exceeding the number of narrow- and wide-spectrum penicillins (39.7% of total antibiotics) recorded by this national healthcare database from 2004 to 2014 [26].

Although these results cannot be directly compared due to the discrepancy between the methods employed to quantify their consumption, they do in some way provide a reference for how frequent the consumption of cefotaxime and third-generation cephalosporins are in general.

In the same way, the infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensible *S. aureus* (MSSA) are frequent in nosocomial environments and a global threat to AMR. Vancomycin remains one of the first-line antibiotics for the treatment of MRSA and oxacillin is the same for MSSA. However, resistance to these agents has emerged in recent years due to the misuse of these antibiotics, especially in empirical treatment scenarios [25].

This study includes the COVID-19 pandemic time frame, 2020–2021; one of the main consequences of this pandemic was the imbalance associated with antimicrobial use, probably due to our initially poor knowledge of the burden of the disease. One important finding is the rise in the incidence of invasive aspergillosis and candidemia during the pandemic; this observation could account for the rise in fluconazole consumption seen in our hospitals, as well as in acute care hospitals in Catalonia [27].

The results from this study showed that trends in overall antimicrobial consumption and consumption per antimicrobial group in DDD per 100 bed days consistently correlate with trends in DDD per 100 discharges over time (Tables 3 and 4). Differences in trends between the two units of measurement seem to be the result of changes in resource indicators over time. Both bed days and discharges underwent statistically significant decreases during the study period; however, the length of stay remained stable.

Comparing the AMC results with other hospitals in different geographical regions remains a challenge, given the heterogeneity in the methodologies employed in this type of study. Despite this challenge, some similarities were found in the literature.

The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) report from 2022 provided information from 36 countries, territories and areas (CTAs) on the status of AMC surveillance implementation, with data available from 2020 [28,29]. However, interpreting the data from this report becomes a challenge, given the small number of reporting CTAs and the wide variety of AMC data collection methods. Data from 26 CTAs included the analysis of AMC, covering all antimicrobials. The findings showed that antibacterials were the most often consumed. Although antibacterial use varied across CTAs, penicillin consumption was 34% of total antibacterial use, with its the highest rate a median of 7.1 (range, 1.0–24.2 DDD) per 1000 inhabitants per day. Third-generation cephalosporins (J01DD) were the most prescribed subgroup within cephalosporins in 12 CTAs, with a median relative consumption of 43% (range, 9–99%). Systemic antifungals (J02, D01B) and antivirals (ATC J05) were reported by 12 CTAs, with median values of 0.9 (range 0.03–3.35) and 1.7 (range, 0.8–3.36) DDD per 1000 inhabitants per day, respectively [28,29].

According to the data from the 2022 Annual Epidemiological Report by the European Centre for Disease Prevention and Control, the reported penicillins had the highest antibiotic use in eighteen Europeans countries, while cephalosporins and other beta-lactams had the highest use in nine countries [30]. From 2013 to 2022, there were statistically significant increases in the hospital AMC of tetracyclines, sulfonamides and trimethoprim,

as well as other antibacterials. However, penicillin, cephalosporins and other beta-lactams, macrolides, lincosamides and streptogramins had no significant trend changes.

In addition to the WHO's world report and the European data, a study was conducted in 2019 across Latin America. This study showed highly variable rates of AMC, from 1.91 DDD/1000 inhabitants in private institutions in Paraguay to 36.26 DDD/1000 inhabitants in Argentina. Furthermore, this study demonstrated that penicillin was the most consumed group in all countries included, except Paraguay, while macrolides and lincosamides were ranked second across the board [17].

In our analysis, we included information from specific regions and countries. For example, in Catalonia, a region of Spain with 7.5 million inhabitants, the most frequently used groups of antibiotics in the period from 2007 to 2019 were penicillin (J01C), quinolones (J01M), cephalosporins (J01DB, J01DC, J01DD and J01DE), other antibiotics (J01X) and carbapenems (J01DH). In 2009, these antibiotic groups represented 88.9% of the total antibiotics consumed in this region. Additionally, a study conducted in México in secondary- and tertiary-care-level hospitals during 2016 and 2017, where AMC was quantified using DDD/100 occupied bed days, showed that the antimicrobials with higher consumption rates were cephalosporines, carbapenems and vancomycin [31].

A study conducted between 2012 and 2020 in Gansu, China, found the three antibiotics most often consumed during this period; first, accounting for 45.15% of AMC, was the penicillin group (J01C), followed by macrolides, lincosamides and streptogramins (J01D), accounting for 31.40%, and then cephalosporins (J01D) at 11.99% [32]. Similarly, our study found that the antibiotics most often used were cephalosporins, penicillins like oxacillin and carbapenems like meropenem.

The Canadian Antimicrobial Resistance Surveillance System Report published in 2022 tracked the country's AMC trend from 2017 to 2021. The report showed that, at the national level, AMC dropped by 26.9%, from 17.0 to 12.5 DDD per 1.00 inhabitants per day. The largest drop was seen at the onset of the COVID-19 pandemic (2020 to 2021). Declines were noted in both the community 27.0% (15.7 to 11.4 DDDs per 1000 inhabitants per day) and the hospital sector 25.3% (1.4 to 1.0 DDDs per 1000 inhabitants per day) [33].

A study from Colombia conducted from 2019 to 2020 demonstrated changes in the reported of DDD/100 occupied beds for specific antibiotics, such as ceftriaxone in ICU wards (2019: 17,584; 2020: 19,857). In addition, the antibiotic consumption reported increased for piperacillin/tazobactam (from 91,606 to 94,076), ertapenem (from 5793 to 6051) and cefepime (26,809 to 38,780) from 2019 to 2020 [34]. Our results are in line with these findings. An increased consumption of carbapenems and penicillin was observed. Despite this, our study is not fully comparable, given a different methodology was employed.

One of the most significant findings is an increase of 7.6% in the Carbapenem use trend, expressed in DDD/100 discharges. This overall increase in the use of carbapenems could lead to the appearance of carbapenem-resistant bacteria. COVID-19 could be partially culpable for this increase, although this is yet to be confirmed. During the early stages of the COVID-19 pandemic, most hospitalized patients with reported, suspected or confirmed secondary bacterial infections were treated with broad-spectrum antibiotics on many occasions, regardless of the severity of the infection [35].

Additionally, a study from Catalonia in reported a worrisome increase in the use of Carbapenem by 88.43%, from 3.37 DDD/100 Patient Days (PDs) to 6.35 DDD/100 PD ($p < 0.001$). This eight-year surveillance study (2008 to 2015) showed a sustained and widespread increase in carbapenem use. This trend is consistent with tendencies observed in other European countries. Meropenem was the most prescribed carbapenem, while the consumption of imipenem-cilastatin showed a downward trend [36].

A peculiar finding was the downward trend in cephalosporins' consumption. A possible explanation is the substitution of cephalosporins with broad-spectrum antibiotics during the pandemic period. Since 2018, antimicrobial stewardship programs have been in operation in the hospitals included in this study, promoting de-escalation and shortened therapies. Furthermore, a downward trend in macrolide consumption was observed.

Also, a downward trend in macrolide consumption was seen with both AAPC indicators: −14.3% DDD/100 bed days and −16.5% DDD/100 discharges. This downward trend may be attributed to the fact that the increase in macrolide resistance minimizes its potential utility in the management of *Streptococcus pneumoniae* infections. Studies suggest that macrolide resistance in *S. pneumoniae* varies widely across different regions, with global resistance rates ranging between 30% and 50% [37].

In contrast, an upward trend was seen with sulfamethoxazole and trimethoprim due to an internal policy on empirical prescriptions established by the AMS program in 2018, which restricted the use of some antibiotics with a high prevalence of resistance such as cephalexin, cephalothin, clindamycin, gentamicin and oxacillin; the prescription of these antibiotics must be justified with an antibiogram or according to pre-existing protocols.

Also, a 9.4% increase in quinolones' DDD/100 discharges has been reported. Quinolones are often used because of their high bioavailability and broad-spectrum antimicrobial activity. In contrast, a study of the trends and patterns of antimicrobial consumption in Japan from 2004 to 2016 indicates that the consumption of oral quinolones has stabilized since 2012, probably due to an increase in fluroquinolone resistance in *Escherichia coli*, which might have restricted its use for urinary tract infections in recent years [25].

Our antimicrobial consumption trend analysis also reported a 2% average increase in DDD/100 discharges for vancomycin; as explained above, this antibiotic is widely used as first-line option for MRSA and *Clostridiodes difficile* infections, which are important healthcare-associated infections in our hospitals [38].

An additional analysis of antimicrobial consumption trends using the AWaRe classification was performed. Firstly, the watch group is the most prominently consumed; this is because agents like cefotaxime, ceftazidime, quinolones and carbapenems are included in this category (Figure 5). On the other hand, there was found to be an average increase of 9.2% ($p = 0.016$) in consumption mostly in the reserve group. An increase in the reserve group is worrying because these antibiotics may be related to the emergence of AMR. In hospitals this could indicate inadequate prescribing; however, it is important to note that these agents were frequently used during the COVID-19 pandemic, which could account for their upward trend. Close monitoring for timely stewardship interventions is needed for the watch and reserve groups [38].

Moreover, an increasing trend in the consumption of azole antifungals was reported. This finding is highly concerning, especially considering the implications of antifungals concerning the morbidity and mortality of mycoses. The clinical consequences of this resistance are observed in treatment failures and changes in the prevalence of fungal species like *Candida auris*, which is spreading at an alarming rate throughout United States healthcare facilities and is considered an "urgent antimicrobial resistance threat" by its health agency [39].

It is important to mention that this study is not without limitations. Firstly, we cannot be certain that the prescribing practices observed were representative of other hospitals of lower complexity in Costa Rica. Additionally, the results could have been influenced by the COVID-19 pandemic, like other studies. Furthermore, other analyses of hospitals of different complexity, other reference hospitals and private institutions and stratifying services into ICU, medical and surgical services would improve the quantity of the data obtained and allow more robust conclusions to be formulated. Moreover, in future studies, other statistic tests could be used to analyze antimicrobial consumption trends, such as the Kendall–Mann. One of the most reported methodologies in the literature used to monitor AMC at a hospital level is the Point Prevalence Survey of hospital antibiotic use. Even though these surveys are also useful for identifying AMS priorities, they do not allow for the evaluation of the duration of therapy, and more research is needed to understand current antibiotic prescribing patterns with respect to their duration, because this has been a major driver of inappropriate antibiotic use [40].

Figure 5. Antibiotic consumption according to WHO AWaRe classification, expressed in DDD/100 bed days (**a**) and DDD/100 discharges (**b**), from 2017 to 2021. DDD: defined daily dose; WHO-AWaRe: World Health Organization antibiotic classification (access, watch and reserve).

4. Materials and Methods

4.1. Setting and Study Design

This retrospective observational study was conducted in general tertiary care hospitals in the CCSS. According to the available data, two out of the three hospitals in this group were included: Hospital San Juan de Dios (635 beds) and Hospital México (466 beds). According to Social Security statistics, these hospitals provide care for approximately 85% of the Costa Rican population in terms of medical and surgical services, as well as providing intensive care units (ICUs) and ambulatory care facilities. This study was approved by the Costa Rican Social Security Ethics Committee: CEC-CENTRAL-CCSS (Protocol #R022-SABI-00319).

4.2. Data Collection

Data were collected monthly, starting from January 2017 and ending in December 2021, with the support of the pharmacy department software "Integrated Pharmacy System" (SIFA). Sixty-one antimicrobials for systemic use (J01, J02 and J05), according to the WHO-ATC classification, representing 46 different active principles, were included in the antimicrobial consumption quantification. Pediatric hospital wards, as well as units that do not generate high rates of discharge or occupied bed days (e.g., emergency services) were excluded from the data collection process. A description of the AMC per group and per specific antibiotic was included. Global consumption trends for antimicrobials

groups and according to the WHO AWaRe classification were calculated and expressed in DDD/100 bed days and DDD/100 discharges, employing the WHO ATC-DDD Index 2023 for all five years as the assumed average maintenance dose per day for a drug used for its main indication adults. Before starting the study, responsible pharmacists were trained in WHO ATC-DDD calculation and the usage of the DDD Excel Calculator to guarantee a homogeneous collection of data [8,9].

4.3. Statistics Analysis

Trends in antimicrobial consumption were analyzed using a simple linear regression model to determine potential differences in antimicrobial usage throughout the period of the study. Simple linear regression was used to determine whether there was a significant difference between the calculated coefficient and zero.

The equation for the simple linear regression is

$$Y = \beta 0 + \beta 1X + \varepsilon$$

where Y: antimicrobial consumption rate in DDD/100 bed days or DDD/100 discharges (dependent variable), β0: the intercept (the estimated antimicrobial consumption when X = 0, or the starting point), β1: the slope (the estimated change in antimicrobial consumption per year), X: the year or time (independent variable), and ε: the error term (captures the variability not explained by the linear relationship)

If β1 (the slope) is positive, it suggests an increasing trend in antimicrobial consumption over the 5-year period; if β1 is negative, it indicates a decreasing trend; and if β1 is close to zero and not statistically significant, it suggests no significant trend in consumption over time.

The comparison of DDD, patient days, discharges and average bed days between 2017 and 2021 was carried out using the Wilcoxon signed-rank test.

Additionally, the average annual percent change (AAPC), with 95 percent confidence intervals, for different classes of antibiotics was incorporated into the analysis to measure the average percentage change in AMC over time, combining the year-to-year fluctuations into a single value.

p values of <0.05 were considered statistically significant. Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and SAS Studio OnDemand for Academics (SAS Institute Inc., Cary, NC, USA).

5. Conclusions

Throughout this five-year study, the trends over time in overall antimicrobial consumption and consumption per group in terms of DDD per 100 bed days consistently correlate with the trends in DDD per 100 discharges. In general, for all antimicrobials, after the implementation of ASPs in our hospitals, a downward trend is reported. This is in contrast to the first year of the COVID-19 pandemic, where the AMC shows a general upward trend. According to the WHO AWaRe classification, the watch group accounts for the predominant proportion of consumption in our time series.

When analyzing specific antimicrobial groups, a concerning increase in carbapenem consumption of 7.6%, expressed in DDD/100 discharges, was observed. Moreover, other agents like sulfonamides and trimethoprim showed an increase of 12.6%, while quinolones showed an increase of 9.4% and vancomycin of 2.0%, a pattern possibly explained by the overuse of broad-spectrum antibiotics during the pandemic.

In contrast, a downward trend in the consumption of cephalosporins, −5.8% DDD/100 bed days and −6.0 DDD/100 discharges, and macrolides, −14.3% DDD/100 bed days and −16.5% DDD/100 discharges, was noticed. This decrease is possibly due to AMS interventions established in 2018 in the participating hospitals and due to the increased resistance of specific bacteria like *S. pneumoniae*, for which macrolides used to be one of the first options for treatment. The increasing use of azole antifungals (+10.3% DDD/100 bed days and +12.1% DDD/100 discharges) could lead to the development of fungal

species resistant to the molecules available in our region. Furthermore, it is important to conclude that the comparison between DDD/100 bed days and DDD/100 discharges allows us to enhance our information about antimicrobial exposure in a clinical setting; with DDD/100 bed days it is possible to describe antibiotic exposure at a hospital level, but only DDD/100 discharges can indicate exposure at the patient level. Knowing the consumption of antimicrobials in a healthcare institution is the first step to improving their rational use and reducing bacterial resistance to these drugs. To determine this consumption, measurement standards must be standardized and validated in order to compare this rate within an institution and with other institutions.

Author Contributions: Conception and design: C.F.-B., S.G. and A.R.-E.; analysis and interpretation of data: C.F.-B., S.G., L.E.H.-S., A.R.-E., M.R.-C., L.D.G.-Z. and J.C.C.; drafting of the paper or revision of intellectual content: C.F.-B., S.G., L.E.H.-S., A.R.-E., M.R.-C., L.D.G.-Z. and J.C.C. All authors have read and agreed to the published version of the manuscript.

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1.7 Second study

Association Between Antimicrobials and Pump Proton Inhibitors Consumption with the Incidence of Nosocomial *Clostridiodes difficile* Infection in High Complexity Hospitals in Costa Rica

Fernández-Barrantes, C.; Ramos-Esquivel, A.; Hernández-Soto, L.E.; Ramírez-Cardoce, M.; Garro-Zamora, L.D.; Cordero, J.C.; Grau, S. Association Between Antimicrobials and Pump Proton Inhibitors Consumption with the Incidence of Nosocomial *Clostridiodes difficile* Infection in High Complexity Hospitals in Costa Rica. *Antibiotics* **2025**, *14*, 350. https://doi.org/10.3390/antibiotics14040350

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Article

Association Between Antimicrobials and Pump Proton Inhibitors Consumption with the Incidence of Nosocomial *Clostridiodes difficile* Infection in High Complexity Hospitals in Costa Rica

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Abstract: Background: Exposure to antimicrobials and Proton Pump Inhibitors (PPIs) are modifiable risk factors for nosocomial *Clostridiodes difficile* infection (CDI). We investigated the association between these agents and nosocomial CDI over five years. **Methods**: Nosocomial CDI from January 2017 to December 2021 were included. Consumption trends were analyzed using a simple linear regression model. A correlation analysis was performed using Spearman's test in two ways: without a time interval and with 1-month interval matching. An interrupted time-series method to evaluate the impact of three key temporal breakpoints on CDI incidence rate was performed using the Poisson regression model. **Results**: A downward trend for cephalexin, ceftriaxone, clindamycin, gentamicin, macrolides, metronidazole, and penicillin sodium was identified. In contrast, an upward trend was recognized for amoxicillin, ceftazidime/avibactam, ertapenem, fluconazole, ketoconazole, levofloxacin, and tigecycline. Among the antimicrobials that showed a positive association between consumption and the incidence of CDI are clindamycin and cephalosporins after immediate consumption. Moreover, macrolides and metronidazole presented a positive correlation, in both immediate and delayed consumption. PPIs consumption did not show changes and was not associated with nosocomial CDI incidence. The interrupted time series analysis showed no changes at the breakpoints selected. **Conclusions**: Consumption of clindamycin, cephalosporins, and macrolides showed positive association with CDI, despite having a downtrend in consumption. Specific events, such as the COVID-19 pandemic and the implementation of ASP, have had no correlation with CDI. Further analysis is required in Latin America to advance our understanding of risk factors associated with CDI.

Keywords: *Clostridiodes difficile* infection; antimicrobial consumption; pump proton inhibitors consumption; defined daily doses; antibiotic prescription

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1. Introduction

Clostridiodes difficile (*C. difficile*) is a Gram-positive, anaerobic bacillus that produces spores and toxins. It is commonly found in the intestinal tract of both humans and animals, as well as in environmental reservoirs. Over the past decade, the rate and severity of *C. difficile* infection (CDI) has risen globally, making it an increasingly major concern among hospital-acquired infections [1]. According to the United States Center of Disease Control and Prevention (CDC), *C. difficile* is considered an antibiotic resistance "urgent" threat, with nearly half a million cases of CDI per year. Out of these, around 30,000 patients will succumb to the illness, costing an estimated USD 5 billion [2]. In 2004, the hypervirulent *C. difficile* polymerase chain reaction (PCR) ribotype 02/toxinotype III was first detected in Canada, the United States, Europe, and in several other countries [3]. CDI presents a wide variety of symptoms, ranging from asymptomatic patients to mild diarrhea, and in certain cases, life-threatening conditions such as pseudomembranous colitis and toxic megacolon [4].

Acquiring CDI involves various risk factors, including exposure of toxigenic strains, prior use of any antimicrobial agent, duration of antimicrobial therapy, and the use of gastric acid suppressants. Additional contributing factors include low host serum immunoglobulin levels, reduced colonic IgA production, advanced age, and severity of any pre-existing conditions. Risk factors can be divided into two main categories: host risks, as previously mentioned, and environmental risks, such as deficient cleaning measures (poor cleaning of contaminated surfaces and hand hygiene methods after contact with patients) and proximity to symptomatic patients, among others [5]. Patients are exposed to the bacteria's spores through contact with the hands of health personnel, or through the hospital's environment. In patients with risk factors or presenting a disrupted gut microbiota, *C. difficile* can germinate, proliferate, and secrete toxins, which are responsible for the development of the above symptoms [6].

The use of antimicrobials remains the most important modifiable risk factor for CDI. Ampicillin, amoxicillin, cephalosporins, clindamycin, and fluoroquinolones are the antibiotics that are most often associated with the disease, which also happen to be classified as high-risk antibiotics. However, almost all antimicrobials have been associated with CDI at a molecular, patient, and population level [7]. Despite their prevalence, the degree to which various antimicrobial classes associate with the risk of CDI is not fully understood and could potentially be of interest when making effective stewardship recommendations [8]. Evidence suggests that 60% of inpatients receiving antibiotics are, on average, more likely to acquire CDI, with risk increasing over time with augmented prescription of antimicrobials [9]. Furthermore, a decrease in antimicrobial consumption, due to antimicrobial stewardship interventions that prioritize the limitation of high-risk antibiotics, resulted in a lower incidence of CDI at a hospital level [10].

Concerns have been raised about the potential long-term effects of Proton pump inhibitors (PPIs). There is evidence of their role in predisposition in both *C. difficile* colonization and infection. It is often theorized that a reduction of gastric acid secretion increases the risk of gut microbiota composition; however, the verdict remains ambiguous on this topic [11].

On March 2020, the World Health Organization (WHO) declared COVID-19 a global pandemic, posing a significant challenge to health systems worldwide, affecting not only their ability to respond to the new disease but also their capacity to maintain essential health services for the general population. Among these challenges, hospitals faced a rise in healthcare-associated infections, with CDI being particularly significant because of its impact on treatment outcomes for patients with COVID-19 and CDI coinfections [12,13].

The gastrointestinal tract (GI) is susceptible to COVID-19 infection and can serve as an alternative route for viral transmission and pathogenesis. GI manifestations of COVID-19 are linked to severe disease outcomes and increased mortality across all age groups, particularly among elderly patients. In hospitalized COVID-19 patients, the use of empiric antibiotics for microbial infections, along with experimental antiviral and immunomodulatory treatments, may elevate the risk of antibiotic-associated diarrhea and CDI [14].

Most of the scientific evidence originates from first world regions such as the United States and Europe. This leads to a region-wide pause in published data in less economically developed regions, such as Latin America, thus causing gaps in available knowledge for treating physicians. Available studies from Latin America have focused predominantly on CDI incidence rates, and limited data are available on risk factors associated with the ailment or its consequences (such as mortality and healthcare utilization) [15].

Globally, the CDI incidence rate rages from 1.1 to 631.8 per 100,000 population per year, [16], while in the European Union in the year 2020, the mean incidence of healthcare-associated CDI was 25.8 cases per 100,000 patients [17] and in the United State, CDI incidence rates in 2021 were 110 cases per 100,000 [18]. In Latin America, a study conducted in Brazil in 2021 found a CDI incidence rate of 92 cases per 100,000 patients; however, there is a lack of information on CDI incidence in Central America and the Caribbean [19].

The incidence of CDI is an important outcome, often evaluated following antimicrobial stewardship program (ASP) interventions, due to the high risk of CDI attributed to broad spectrum antibiotic therapy [20]. However, there are differences between *C. difficile* strains and their antimicrobial susceptibility depending on the region or country.

Since 2003, the rise in hypervirulent strains such as NAP1 and NAP9 has coincided with an increase in CDI incidence, severity, complications, recurrence, and mortality. NAP1 strains are characterized by enhanced sporulation and greater resistance to antimicrobials, particularly fluoroquinolones. Meanwhile, NAP9 strains produce the TcdB variant, which is capable of inducing a distinct cytopathic effect [21]. A *C. difficile* outbreak in a major Costa Rican hospital in 2009 was primarily driven by the hypervirulent NAP1 strain (45%). This strain demonstrate resistance to ciprofloxacin, moxifloxacin, and levofloxacin. Although NAP9 and other classical nosocomial strains were also identified, they were present in smaller proportions. Since this outbreak, the distribution of *C. difficile* genotypes in other Costa Rican hospitals has not been documented [22].

The role of antimicrobial consumption is usually well described at patient level; however, there is no conclusive evidence on risk factors at a ward-wide level. As a result, analysis was conducted throughout this ecological study, examining whether the evolution in nosocomial CDI rates in Tertiary Care Hospitals in Costa Rica could have any relationship with antimicrobial and PPIs consumption, expressed in defined daily doses (DDD)/100 bed days. Two different analysis methods were used. First, a correlation test during a five-year series without a time interval and with 1-month interval was conducted that was then followed by the second method, which uses time series analysis at different breakpoints of interest, including after ASP implementation and during the COVID-19 pandemic.

2. Results

2.1. Incidence of Clostridiodes difficile Infection

Between January 2017 and December 2021, a total of 701 nosocomial CDI and 1,636,688 bed-days were identified, proceeded by monthly CDI rate measurement. During the study period, the incidence of nosocomial CDI was lower in the pandemic years (2020 and 2021), reporting a mean of 0.44 infections per 100 bed days and 0.42 infections per 100 bed days, respectively. The lowest infection rate was found in September 2020

with 0.009 CDI per 100 bed days, while the highest rate was reported in 2018 with a total 0.67 infections per 100 bed days. The month with the highest rate was May 2018, reporting 0.095 per 100 bed days. The mean incidence of CDI during this time series was 0.043 infections per 100 bed days. Nosocomial CDI trend per period shows no statistically significant changes; hospital onset CDI in January 2019 is 0.022 (−13.25 to 13.29) $p = 0.99$, in March 2020 is 0.30 (−14.98 to 14.30) $p = 0.96$, and in September 2020 is −1.56 (24.28 to 21.15) $p = 0.89$. Figure 1 shows the evolution of rates of nosocomial CDI for the study cohort.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2017	0.05	0.031	0.014	0.026	0.053	0.05	0.034	0.057	0.063	0.024	0.03	0.056
2018	0.054	0.066	0.035	0.054	0.095	0.06	0.059	0.049	0.059	0.052	0.042	0.045
2019	0.044	0.083	0.035	0.034	0.035	0.081	0.024	0.06	0.029	0.017	0.045	0.054
2020	0.07	0.071	0.031	0.047	0.067	0.013	0.019	0.053	0.009	0.022	0.023	0.012
2021	0.024	0.033	0.031	0.025	0.031	0.03	0.043	0.038	0.043	0.053	0.036	0.038

Figure 1. Heatmap showing the incidence of nosocomial *Clostridiodes difficile* infection in Tertiary Care Hospitals in Costa Rica from January 2017 to December 2021, expressed in cases per 100 bed-days. Colors indicate the intensity of data, with warmer colors representing higher values and cooler colors representing lower values.

2.2. *Antimicrobial Consumption*

A total of 1,806,292 DDDs of antibiotics, including 892,616 DDDs of high-risk antibiotics (penicillins, cephalosporins, fluoroquinolones, carbapenems, and clindamycin), were distributed during the five-year series. Figure 2 shows antibiotic consumption trends from 2017 to 2021 in certain selected groups. Antimicrobial consumption trends of most important groups calculated in DDD/100 bed days and DDD/100 discharges from participating hospitals in this same study period were previously published [23]. When analyzing consumption per antibiotic group, cephalosporins were presented as the most used antibiotic group, followed by penicillins and carbapenems.

Nevertheless, the analysis of antibiotic consumption using the World Health Organization Access, Watch and Reserve (WHO AWaRe) classification shows no statistically significant changes (results are presented in Table 1). Consequentially, a more specific statistical analysis was performed, which included individual antimicrobial consumption trends combined with PPIs consumption.

Table 1. Antimicrobial consumption trends according to WHO AWaRe classification, expressed in DDD/100 bed days and DDD/100 discharges.

Total	2017	2018	2019	2020	2021	% AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
ACCESS	621.18	553.66	539.78	748.08	519.41	−4.4	−47.6 to 45.0	−0.91	0.97
WATCH	746.69	723.13	752.68	1079.66	623.95	−4.4	−55.3 to 56.4	11.1	0.81
RESERVE	58.42	35.33	54.68	92.34	55.34	−1.3	−82.8 to 104.8	5.08	0.34

WHO AWaRe: World Health Organization antibiotic classification (access, watch and reserve); DDD: defined daily dose; % AAPC: average annual percent change; 95% CI: 95% confidence intervals; Trend β1: the estimated change in antimicrobial consumption per year.

A significant decrease consumption trend in terms of DDD/100 bed days was reported as a group for Macrolides −14.3% AAPC (average annual percent change) 2017–2021, trend −6.54 ($p = 0.005$) and as individual antibiotics for: Cephalexin −0.23% AAPC 2017–2021, trend −15.99 ($p < 0.0001$), Ceftriaxone −0.25% AAPC 2017–2021, trend −0.010 ($p < 0.0001$), Clindamycin −0.16% AAPC 2017–2021, trend −3.48 ($p < 0.0001$), Gentamicin −0.12% AAPC

2017–2021, trend −1.71 ($p < 0.0001$), Metronidazole −0.12% AAPC 2017–2022, trend −6.21 ($p < 0.0001$), and Penicillin sodium −0.14% AAPC 2017–2021, trend −5.56 ($p < 0.0001$).

Antimicrobial	2017	2018	2019	2020	2021
Acyclovir	4.08	5.47	7.05	19.68	8.61
Amikacin	53.68	41.5	52.41	77.95	61.6
Amoxicillin	9.59	11.45	9.99	16.27	13.84
Ampicillin sulbactam	0.02	0.6	0.14	10.52	1.01
Ampicillin	25.98	23.05	29.78	46.44	29.17
Anidulafungin	41.22	28.81	38.47	69.4	28.02
Azithromycin	0.02	0.01	4.17	6.84	1.97
Aztreonam	2.85	1.35	0.04	1.47	7.37
Caspofungin	0.03	1.47	2.89	10.52	3.31
Cefalexin	89.6	89.34	63.83	51.83	28.39
Cefalotin	37	32.4	28.81	43.88	22.22
Cefotaxime	223.02	217.85	210.53	275.23	167.82
Ceftaroline	0	0.44	2.77	2.94	1.45
Ceftazidime	80.99	64.99	62.13	108.61	70.3
Ceftazidime +avibactam	0	0	0.17	1.77	5.89
Ceftolozane + tazobactam	0	0.87	0.83	1.47	0
Ceftriaxone	0.04	0.02	0.03	0	0
Ciprofloxacin	26.95	30.56	22.07	33	25.45
Clarithromycin	19.96	38.31	16.97	19.25	8.86
Clindamycin	30.44	23.67	23.03	20.56	14.55
Doxycycline	14.97	13.24	12.61	15.02	12.06
Ertapenem	12.61	25.45	34.17	66.42	46.77
Fluconazole	103.82	101.22	101.73	147.86	147.18
Gancyclovir	11.64	6.74	7.08	15.58	2.58
Gentamicin	15.94	15.1	11.35	10.99	9.41
Imipenem + cilastatin	0.42	0.13	0.91	1.84	0.08
Itraconazole	6.23	2.54	8	5.14	3.49
Ketoconazole	0.02	0.21	0.19	0.79	0.42
Levofloxacin	41.06	25.46	37.31	80.04	58.65
Linezolid	46.9	28.36	48.08	77.36	35.42
Meropenem	130.24	120.78	137.42	190.36	102.55
Metronidazole	63.09	60.33	47.58	52.32	36
Nitrofurantoin	6.72	7.98	7.55	8.41	6.8
Oxacillin	145.52	114.92	117.1	178.03	149.14
Penicillin benzathine	0.08	0.11	0.03	0.16	0.08
Penicillin sodium	54.12	42.16	34.97	38.9	27.93
Piperacillin - tazobactam	28.72	26.26	41.12	49.16	0.08
Polymyxin	8.49	3.72	2.69	5.48	3.48
Rifampicin	6.63	6.63	4.14	7.44	1.76
Tigecycline	0.17	0.59	0.11	1.84	1.72
Sulfamethoxazole and trimethoprim	74.42	77.82	100.6	176.83	107.19
Vancomycin	176.02	166.4	181.72	241.48	139.66
Valgancyclovir	7.73	6.28	5.22	9.62	7.68
Voriconazole	6.45	1.82	14.29	25.55	12.55

Figure 2. Heatmap of antimicrobial use at participating hospitals from 2017 to 2021, consumption is expressed in DDD/100 bed-day. Colors indicate the intensity of data, with warmer colors representing higher values and cooler colors representing lower values DDD: Defined daily doses.

In contrast, a significant upward trend was identified for the following antimicrobials: Amoxicillin 0.14% AAPC 2017–2021, trend 1.22 $p = 0.01$, Ceftazidime/avibactam 2.9% AAPC 2017–2021, trend 1.35 $p = 0.0004$, Ertapenem 0.50% AAPC 2017–2021, trend 10.92 $p = 0.0006$, Fluconazole 0.11% AAPC 2017–2021, trend 13.33 ($p = 0.0003$), Ketoconazole 3.02% AAPC 2017–2021, trend 0.13 $p = 0.01$, Levofloxacin 0.24% AAPC 2017–2021, trend 8.97 $p = 0.04$, and Tigecycline 4.33% AAPC 2017–2021, trend 0.43 ($p = 0.001$). Table 2 summarizes the antimicrobial consumption trends recognized in the time series.

Table 2. Antimicrobial consumption trends from 2017 to 2021, expressed in DDD/100 bed days.

Total	2017	2018	2019	2020	2021	% AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
Antibiotics	30.36	27.92	28.67	40.86	25.51	−3.42	−32.78 to 25.94	0.32	0.84
Aminoglycosides	69.62	56.59	63.76	88.93	71.01	3.00	−18.50 to 24.50	3.51	0.24
Amikacin	53.68	41.50	52.41	77.95	61.60	0.08	−0.49 to 0.64	5.22	0.08
Gentamicin	15.94	15.10	11.35	10.99	9.41	−0.12	−0.28 to 0.04	−1.71	**<0.0001**
Cephalosporins	430.66	405.92	369.09	485.73	296.08	−5.80	−27.80 to 16.20	−18.93	0.29
Cephalexin	89.60	89.34	63.83	51.83	28.39	−0.23	−0.53 to 0.07	−15.99	**<0.0001**
Cephalothin	37.00	32.40	28.81	43.88	22.22	−0.05	−0.72 to 0.62	−1.81	0.40
Cefotaxime	223.02	217.85	210.53	275.23	167.82	−0.03	−0.49 to 0.42	−5.30	0.61
Ceftaroline	0.00	0.44	2.77	2.94	1.45	1.21	−3.14 to 5.56	0.53	0.06
Ceftazidime	80.99	64.99	62.13	108.61	70.30	0.04	−0.74 to 0.82	2.22	0.67
Ceftazidime + avibactam	0.00	0.00	0.17	1.77	5.89	2.93	−4.15 to 10.02	1.35	**0.0004**
Ceftolozane + tazobactam	0.00	0.87	0.83	1.47	0.00	−0.07	−1.22 to 1.09	0.059	0.73
Ceftriaxone	0.04	0.02	0.03	0.00	0.00	−0.25	−1.28 to 0.78	−0.010	**<0.0001**
Carbapenems and Monobactams	146.13	147.72	172.54	260.09	156.77	6.60	−21.7 to 35.0	13.36	0.27
Aztreonam	2.85	1.35	0.04	1.47	7.37	9.57	−18.44 to 37.57	0.91	0.18
Ertapenem	12.61	25.45	34.17	66.42	46.77	0.50	−0.47 to 1.48	10.92	**0.0006**
Imipenem + cilastatin	0.42	0.13	0.91	1.84	0.08	1.34	−3.79 to 6.48	0.10	0.61
Meropenem	130.24	120.78	137.42	190.36	102.55	0.00	−0.57 to 0.57	1.41	0.87
Fluoroquinolones	68.01	56.02	59.38	113.04	84.09	13.20	−27.10 to 53.50	8.91	0.08
Ciprofloxacin	26.95	30.56	22.07	33.00	25.45	0.03	−0.54 to 0.60	−0.056	0.96
Levofloxacin	41.06	25.46	37.31	80.04	58.65	0.24	−0.89 to 1.37	8.97	**0.04**
Macrolides	50.42	61.99	44.17	46.65	25.39	−14.30	−40.00 to 11.40	−6.54	**0.005**
Azithromycin	0.02	0.01	4.17	6.84	1.97	103.86	−227.27 to 434.98	1.07	0.11
Clarithromycin	19.96	38.31	16.97	19.25	8.86	−0.01	−1.12 to 1.10	−4.12	0.09
Penicillins	264.03	218.55	233.13	339.47	221.26	0.00	−26.40 to 26.40	3.53	0.80
Amoxicillin	9.59	11.45	9.99	16.27	13.84	0.14	−0.44 to 0.72	1.22	**0.01**
Ampicillin + sulbactam	0.02	0.60	0.14	10.52	1.01	25.37	−31.01 to 81.74	1.19	0.30
Ampicillin	25.98	23.05	29.78	46.44	29.17	0.09	−0.57 to 0.75	2.97	0.17
Oxacillin	145.52	114.92	117.10	178.03	149.14	0.04	−0.49 to 0.57	7.03	0.29
Penicillin benzathine	0.08	0.11	0.03	0.16	0.08	0.87	−2.88 to 4.62	0.0041	0.75
Penicillin sodium	54.12	42.16	34.97	38.90	27.93	−0.14	−0.42 to 0.14	−5.56	**<0.0001**
Piperacillin + tazobactam	28.72	26.26	41.12	49.16	0.08	−0.08	−1.14 to 0.98	−3.43	0.49
Sulfamethoxazole + trimethoprim	74.42	77.82	100.60	176.83	107.19	15.90	−20.30 to 52.10	16.45	0.06
Tetracyclines	15.14	13.83	12.72	16.86	13.78	−0.80	−18.00 to 16.40	0.031	0.94

Table 2. *Cont.*

Total	2017	2018	2019	2020	2021	% AAPC 2017–2021	AAPC 95% CI	Trend (β1)	*p* Value
Doxycycline	14.97	13.24	12.61	15.02	12.06	−0.04	−0.31 to 0.22	−0.40	0.23
Tigecycline	0.17	0.59	0.11	1.84	1.72	4.33	−7.97 to 16.63	0.43	**0.001**
Clindamycin	30.44	23.67	23.03	20.56	14.55	−0.16	−0.35 to 0.03	−3.48	**<0.0001**
Linezolid	46.90	28.18	47.76	76.56	34.61	1.80	−41.70 to 45.30	2.47	0.62
Nitrofurantoin	6.72	7.98	7.55	8.41	6.80	0.01	−0.26 to 0.28	0.058	0.77
Polymyxin	8.49	3.72	2.69	5.48	3.48	−0.04	−1.20 to 1.12	−0.82	0.12
Rifampicin	6.63	6.63	4.14	7.44	1.76	−0.09	−1.14 to 0.97	−0.89	0.09
Vancomycin	176.02	166.40	181.72	241.48	139.66	−1.60	−25.40 to 22.20	0.23	0.98
Antifungals	26.30	22.68	27.60	43.21	32.49	4.31	−28.22 to 36.84	3.29	0.05
Anidulafungin	41.22	28.81	38.47	69.40	28.02	0.06	−0.94 to 1.06	1.41	0.76
Caspofungin	0.03	1.47	2.89	10.52	3.31	12.73	−24.75 to 50.21	1.56	0.08
Fluconazole	103.82	101.22	101.73	147.86	147.18	0.11	−0.26 to 0.48	13.33	**0.0003**
Itraconazole	6.23	2.54	8.00	5.14	3.49	0.22	−1.84 to 2.28	−0.28	0.63
Ketoconazole	0.02	0.21	0.19	0.79	0.42	3.02	−4.32 to 10.37	0.13	**0.01**
Metronidazole	63.09	60.33	47.58	52.32	36.00	−0.12	−0.41 to 0.17	−6.21	**<0.0001**
Voriconazole	6.45	1.82	14.29	25.55	12.55	1.60	−4.06 to 7.27	3.59	0.06
Antivirals	4.69	3.70	3.87	8.98	3.77	−4.27	−74.42 to 65.88	−0.34	0.57
Acyclovir	4.08	5.47	7.05	19.68	8.61	0.46	−1.09 to 2.02	2.32	0.10
Gancyclovir	11.64	6.74	7.08	15.58	2.58	0.00	−1.40 to 1.40	−0.92	0.49
Valgancyclovir	7.73	6.28	5.22	9.62	7.68	0.07	−0.75 to 0.89	0.32	0.47
Proton pump inhibitors	356.21	355.88	498.71	667.63	315.82	0.05	−0.62 to 0.73	23.09	0.56

DDD: defined daily dose; % AAPC: average annual percent change; 95% CI: 95% confidence intervals; Trend β1: the estimated change in antimicrobial consumption per year; statistically significant *p* values are in bold.

PPIs do not show statically significant changes during the study period. Figure 3 shows the PPIs consumption trend.

Time	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2017	411.40	285.78	626.49	364.03	280.31	252.59	452.07	323.20	251.82	330.15	388.06	308.62
2018	275.66	563.27	318.02	222.52	230.87	240.79	305.47	300.98	517.02	402.62	271.25	622.08
2019	434.26	1368.06	403.22	494.49	346.85	356.28	367.64	264.27	551.72	257.76	271.15	868.81
2020	1236.77	916.35	522.12	858.79	1193.73	457.99	522.36	924.75	339.33	345.30	267.88	426.23
2021	374.32	502.70	717.62	421.45	164.98	201.72	301.43	208.47	196.11	194.91	224.75	281.42

Figure 3. Heatmap of Proton Pump Inhibitors use at participating hospitals in Costa Rica from 2017 to 2021, consumption expressed in DDD/100 bed days. Colors indicate the intensity of data, with warmer colors representing higher values and cooler colors representing lower values. DDD: Defined daily doses.

2.3. Correlation Between CDI Rates with Antimicrobial Consumption and PPI

Total antimicrobial and PPIs consumption was not significantly associated with nosocomial CDI incidence. Moreover, according to the WHO AWaRe analysis, there was no statistically significant correlation identified.

The following antibiotics showed a positive correlation with a statistically significant downtrend for CDI, in both immediate and delayed consumption: cephalexin ($p = 0.03$ and 0.002), macrolides ($p = 0.0082$ and $p = 0.0023$), metronidazole ($p = 0.0002$ and $p = 0.01$), clindamycin with immediate consumption effect ($p = 0.0055$), and gentamicin with 1-month consumption delayed effect ($p = 0.02$).

In addition, some antibiotics also show positive correlation for CDI, without any statically significant consumption trend. In this scenario, cephalothin describes positive correlation for immediate ($p = 0.028$) and delayed consumption ($p = 0.0097$), and clarithromycin ($p = 0.0061$ and $p = 0.006$) and cephalosporins as a group with immediate consumption ($p = 0.035$).

According to the literature, cephalosporins (such as cephalexin and cephalothin) and clindamycin are considered high-risk antibiotics for CDI development [7,15]. We observed a decrease in the consumption of these antimicrobials (cephalexin and clindamycin). There is a positive correlation for CDI, demonstrating the strong association of these antibiotics as a risk of infection.

On the other hand, antifungals and fluoroquinolones consumption shows a negative statically significant correlation for CDI with 1-month consumption delayed effect ($p = 0.04$ and $p = 0.0051$, respectively) without any changes in antibiotic consumption trends.

Negative statistically significant correlations were also reported for the immediate consumption effect of acyclovir ($p = 0.01$) and voriconazole ($p = 0.0024$). These antimicrobials show independent correlation with consumption trends. Furthermore, ceftazidime/avibactam shows negative correlation with both immediate ($p = 0.013$) and 1-month delayed consumption effect ($p = 0.002$). Finally, levofloxacin shows a negative correlation with immediate effect ($p = 0.0001$). Ceftazidime/avibactam and levofloxacin consumption present a statistically significant upward trend during our time series.

Table 3 presents the complete correlation analysis results for every antimicrobial group and individual antimicrobial without time interval matching and with 1-month interval matching.

Table 3. Correlation analysis between antimicrobial and proton pump inhibitors consumption and the incidence of nosocomial *Clostridioides difficile* without a time interval and with a 1-month interval matching.

Class of Antibiotics	DDD Per 100 Bed Days Spearman p Without Time Interval Matching	p Value	DDD Per 100 Bed Days Spearman p with a 1-Month Interval Matching	p Value
AWaRe				
Access	0.07	0.57	0.041	0.75
Reserve	−0.062	0.63	−0.109	0.41
Watch	0.089	0.49	−0.026	0.84
Antivirals				
Antivirals	−0.113	0.38	−0.186	0.15
Acyclovir	−0.305	**0.01**	−0.217	0.09
Gancyclovir	0.08	0.54	−0.0151	0.90
Valgancyclovir	−0.89	0.49	−0.210	0.10
Antifungals				
Antifungals	−0.202	0.12	−0.26	**0.04**
Anidulafungin	−0.070	0.59	−0.20	0.11
Caspofungin	−0.073	0.57	−0.07	0.57
Fluconazole	−0.201	0.12	−0.27	0.03
Itraconazole	0.047	0.72	−0.12	0.34
Ketoconazole	0.058	0.65	−0.076	0.56
Voriconazole	−0.38	**0.0024**	−0.216	0.10
Antibiotics				
Antibiotics	0.07	0.56	0.015	0.90
Aminoglycosides	0.021	0.86	−0.15	0.25
Amikacin	−0.070	0.59	−0.22	0.08
Gentamicin	0.203	0.12	0.28	**0.02**

Table 3. *Cont.*

Class of Antibiotics	DDD Per 100 Bed Days Spearman p Without Time Interval Matching	p Value	DDD Per 100 Bed Days Spearman p with a 1-Month Interval Matching	p Value
Carbapenems	−0.096	0.46	−0.053	0.68
Aztreonam	−0.22	0.08	−0.116	0.37
Ertapenem	−0.077	0.55	−0.074	0.57
Imipenem Cilastatin	0.179	0.17	0.188	0.15
Meropenem	0.00214	0.98	−0.020	0.87
Cephalosporins	0.27	**0.035**	0.14	0.28
Cephalexin	**0.274**	**0.03**	0.383	**0.002**
Cephalotin	0.28	**0.028**	0.334	**0.0097**
Cefotaxime	0.17	0.17	−0.0315	0.81
Ceftazidime/Avibactam	−0.318	**0.013**	−0.38	**0.002**
Ceftaroline	−0.122	0.35	−0.17	0.17
Ceftazidime	0.087	0.50	−0.056	0.67
Ceftolozano/Tazobactam	0.169	0.19	−0.092	0.48
Ceftriaxone	0.024	0.85	0.070	0.59
Macrolides	0.338	**0.0082**	0.39	**0.0023**
Azitromicina	−0.064	0.62	−0.037	0.78
Clarithromycin	0.350	**0.0061**	0.35	**0.006**
Penicilinas	0.063	0.63	0.027	0.83
Amoxicillin	0.03	0.80	−0.10	0.44
Ampicillin Sulbactam	−0.15	0.25	0.09	0.49
Ampicillin	−0.050	0.70	−0.24	0.06
Oxacillin	0.028	0.82	−0.116	0.38
Penicillin sodium	0.176	0.17	0.23	0.07
Penicillin benzathine	−0.154	0.23	0.02	0.86
Piperacillin/Tazobactam	0.196	0.13	0.116	0.37
Fluoroquinolones	−0.055	0.67	−0.35	**0.0051**
Ciprofloxacin	0.208	0.10	0.244	0.06
Levofloxacin	−0.200	0.12	−0.47	**0.0001**
Tetracyclines	0.048	0.71	0.015	0.90
Doxycycline	0.058	0.65	0.057	0.66
Tigecycline	−0.179	0.17	−0.19	0.12
Other antibiotics				
Clindamycin	0.354	**0.0055**	0.128	0.33
Linezolid	−0.123	0.35	−0.117	0.37
Metronidazole	0.460	**0.0002**	0.30	**0.01**
Nitrofurantoin	0.024	0.85	−0.033	0.80
Polymyxin	−0.0120	0.92	0.0007	0.99
Rifampicin	0.072	0.58	0.25	0.05
Sulfamethoxazole and Trimethoprim	−0.207	0.11	−0.25	0.05
Vancomycin	0.0704	0.59	0.050	0.70
Proton Pump Inhibitors	−0.139	0.28	−0.14	0.26

DDD: defined daily dose. Statistically significant p values are in bold.

2.4. Interrupted Time Series Analysis

The correlation analysis of antimicrobial and PPI consumption with the incidence of nosocomial CDI indicates specific positive or negative associations during the five-year time series; however, they do not give information of when these correlations have more impact in the development of nosocomial CDI. For that reason, three interrupted time series analyses were performed using three main breakpoints. The Interrupted time series analysis studied immediate changes in antimicrobial and PPI consumption, as well as trend changes after the breakpoint and their respective relationships with nosocomial CDI rate.

There were no statistically significant changes in any of the three breakpoints. Table A1 (Appendix A) summarizes the segmented Poisson's regression results for this interrupted time series analysis.

3. Discussion

To the extent of our knowledge, this article presents the first ecological study in Latin America that combines the evaluation of changes in nosocomial CDI rates coupled with the consumption of antimicrobials and PPIs, while applying correlative tests and interrupted time series analysis using Poisson's regression model. Throughout the given time frame, no statistically significant changes in nosocomial CDI rates were observed. However, it is noteworthy to point out that there were significant changes in antimicrobial consumption trends, along with the emergence of the COVID-19 pandemic forcing hospitals to adopt infection control measures and strategies to mitigate antimicrobial resistance (e.g., antimicrobial stewardship programs) that were present during the study's timeframe. The epidemiology of nosocomial *C. difficile* infections has changed over the past years in Europe, North America, and Latin America, with substantial changes in disease incidence and in the prevalence of specific strains like NAP1/027, possibly caused by different burdens of risk factors such as antibiotic use, recent hospitalizations, PPIs use, and increasing age [24,25]

A study published in 2019 that examined the global burden of *C. difficile* estimated that the overall incidence of nosocomial CDI was 4.14 per 10,000 patients-days, ranging from 1.67 (0.58–4.84) in Europe, 2.69 (1.32–5.49) in the Western Pacific, and 6.36 (5.53–7.19) in North America [26].

In this study, the mean incidence of CDI was 0.043 infections per 100 bed days, a lower incidence rate than reported in other regions. As an example, a multicentric study in Germany evaluated nosocomial CDI from 2016 to 2020, reporting 0.30 cases/100 patients in 2016 and 0.26 cases/100 patients in 2020. However, given there are no precise data of the incidence rate in Latin America, no conclusion can be drawn about the regional incidence rate [25,27].

The effect of the COVID-19 pandemic on the incidence of CDI has been explored in several studies, demonstrating mixed findings. While some experts suggested that hospitalized COVID-19 patients might be at higher risk for CDI, most research has reported either no significant change or a decline in CDI cases during the early pandemic years, as shown in our study [28]. Several factors may have influenced this trend, including changes in patient management and the suspension of elective surgeries and routine medical care for non-COVID-19 conditions due to strict lockdown measures. Additionally, enhanced infection prevention strategies, such as visitor restrictions, improved hand hygiene, increased use of personal protective equipment, and the isolation of COVID-19 patients in designed areas, may have contributed to this trend [29].

CDI incidence is a significant outcome often evaluated following AMS program intervention. This is due to the high risk of CDI attributed to broad-spectrum antibiotic use. Risk of CDI conferred varies by agent, with fluoroquinolones, clindamycin, and later-generation cephalosporins associated with higher levels of risk [4,20]. Published research estimates that hospitalized patients receiving antibiotics are on average 60% more likely to acquire CDI. Additionally, prolonged antibiotic exposure, as well as large doses, are correlated with a higher risk of infection. The high prevalence of antibiotic use increases hospital environmental contamination, consequently leading the CDI rate to increase [9].

Brown et al. concluded that, relative to a 7-day course of antibiotics, a 14-day course was associated with 27% more risk, whereas a short course was associated with 9% less risk [30]. Similarly, Owens (2007) concluded that antimicrobials that were formerly thought of as posing low to moderate risk were more commonly associated with CDI thanks to fre-

quent exposure and use [5]. Moreover, the concept of high-risk antibiotics is also explained by Lawes et al. [7], who studied the effect of a national plan to reduce community and hospital antibiotic use associated with CDI. Known as the 4Cs (ciprofloxacin/fluoroquinolones, co-amoxiclav, clindamycin, and cephalosporins), alongside broad-spectrum Beta-lactams, carbapenems and macrolides, these antibiotics were linked with increased risk of CDI at molecular, patient, and population levels. They found that limiting the population use of 4C antibiotics reduces selective pressures and is associated with substantial declines in total CDI [7].

Examining the data obtained in this article, the tendency of antimicrobial consumption changed during this time series analysis. The major findings were an upward trend in amoxicillin, ceftazidime/avibactam, ertapenem, fluconazole, ketoconazole, levofloxacin, and tigecycline. Increased prescription of these compounds could translate to higher antimicrobial exposure in patients. There is further evidence suggesting that ward-level antibiotic exposure is the main risk for CDI, with every 10% increase in ward antibiotic increase being associated with a 1.34-fold increase in *C. difficile* infection risk. A high prevalence of antibiotic use would increase environmental contamination and CDI incidence [9].

It is worthy to point out that this study includes data from the COVID-19 pandemic period, which could influence the statistical significance of antibiotic consumption increases. A study of global antibiotic use during the COVID-19 pandemic (from 2020–2022) found a 10% increase in monthly COVID-19 cases was associated with 0.2–0.3% higher sales of cephalosporins, 0.2–0.3% higher sales of penicillins, 0.4–0.6% higher sales of macrolides, and 0.3% higher sales of all four antibiotics combined per 1000 people. Sales of other antibiotics across continents were also positively associated with COVID-19 cases [31]. Furthermore, in 2020, an outbreak of New Delhi metallo-β-lactamase 1 (NDM) was reported in local hospitals. Consequently, the introduction of ceftazidime/avibactam became mandatory, thus increasing the use of these agents.

This increase in antibiotic use can also be partially explained by the treatment of bacterial coinfection amongst COVD-19 patients, as well as the increased workload for infectious disease professionals. This resulted in the interruption of antibiotic stewardship programs. This initial increase in antibiotic consumption was followed by a reported decrease in antibiotic consumption [32]. In this study, a decrease in cephalexin, ceftriaxone, clindamycin, gentamicin, macrolides, metronidazole, and penicillium sodium was reported. Some of the reasons for the downtrend of these molecules during the study period include a decrease in antibiotic use for respiratory infection, which reflects the effectiveness of personal infection protection efforts, and antimicrobial stewardship interventions as well as people's hesitancy of visiting hospitalized patients due to the fear of asymptomatic COVID-19 patients, as well as reduced social activities, e.g., city or country lockdown, which decreases the likelihood of acquiring an infection and increased awareness and infection prevention activities among the public (e.g., enhanced hand hygiene, universal wearing of masks, and social distancing) [32].

In this study's correlation analysis, a positive statistically significant association of CDI with the consumption of gentamicin, cephalosporins (cephalothin, cephalexin), macrolides, clindamycin, and metronidazole were identified. Different classes of medications carry different levels of risk, coupled with the intra-class stratification differences. Included in these differences are compounds such as cephalosporins, clindamycin, penicillin, metronidazole, vancomycin, and fluoroquinolones. It is important to understand that these differences are not fixed but rather evolve according to rapidly changing epidemiology (different strains and different susceptibilities) [3].

Similarly to the findings of our study, the results presented in a study about the global burden and trend of *C. difficile* and its association with world antibiotic consumption in the

period 1990–2019 demonstrated a positive association for CDI, with different risk stratification for carbapenems (second highest risk association), cephalosporins, clindamycin (highest risk association), fluoroquinolones, broad-spectrum penicillins, and macrolides; in contrast, trimethoprim-sulfamethoxazole has the lowest risk association [26].

In addition, data from the European Centre for Disease Prevention and Control (ECDC) reported that cephalosporins were the second most used subgroup of antibiotics in hospitals at the European level in 2023. Also, according to this report, carbapenems and third and fourth generation cephalosporins are antibiotics that are the most strongly associated with nosocomial CDI, while a modest association was observed for fluoroquinolones, which is similar to our main finding [12].

According to a study conducted in Colombia, cephalosporins are among the antibiotics more commonly associated with CDI development due to their wide effect on gut microbiota. The risk associated with cephalosporins decreases from third/fourth, to second, to first generation [33]. A strong positive correlation was identified for cephalosporins and clindamycin, even though these antibiotics show a downtrend in consumption. A systematic review and meta-analysis from 2020 concluded that carbapenems and third- and fourth generation cephalosporins remain the most strongly associated with CDI; modest associations were observed for fluoroquinolones, clindamycin, second-generation cephalosporins, and Beta-lactamase inhibitors in combination with penicillin antibiotics [4]. As for gentamicin, there is evidence of a positive correlation between this antibiotic and CDI according to pharmacovigilance reports from the WHO medication monitoring center [34].

A study from Argentina and Mexico concluded that neither cephalosporins nor fluoroquinolones were found in association with CDI [35]. Similarly, in this study's analysis, fluoroquinolones did not show a positive correlation with CDI. In fact, they showed a statistically significant negative association, which is not dissimilar to that reported for ceftazidime-avibactam, levofloxacin, acyclovir, antifungals, and voriconazole. This could reflect possible bias due to underdiagnosis in certain periods of the COVID-19 pandemic due to changes in healthcare priorities.

This study did not identify statistically significant changes in PPI consumption trends and did not find correlations with CDI incidence, supporting the evidence that suggests a weak association between PPIs use and risk of CDI. In local hospitals, there are protocols and restrictions for PPIs use, which could explain why restrictions on PPIs use could lead to a negative association of these agents with *C. difficile*. However, the literature states that gastric acid suppressants have long been identified as predisposing patients to CDI. Vegetative forms of the bacteria survive on surfaces and could be ingested by patients. Survival of these acid-sensitive vegetative forms in the stomach could be facilitated by two main factors: suppression of gastric acid production by acid-suppressive medications, and presence of bile salts in gastric contents of patients on acid-suppressive therapy. Moreover, PPI use can delay gastric emptying and predispose patients to bacterial overgrowth with associated high intragastric bile salts, which could trigger spore germination in the stomach [36].

Finally, our time series analysis did not show specific changes at predefined breakpoints, such as the beginning of the COVID-19 pandemic and the establishment of ASP programs, meaning that in the present scenarios, these events had no direct relation with CDI incidence.

Although ASP has been associated with a reduction in nosocomial CDI incidence, ASPs that target only specific broad-spectrum antimicrobials can miss opportunities to reduce CDI and antimicrobial resistance in the hospital setting. Also, the overuse of other unnecessary antimicrobials like carbapenems could lead to other nosocomial infections. During the COVID-19 pandemic, ASPs were limited due to other healthcare challenges;

consequently, a significant increase in antimicrobial consumption could lead to higher rates of antimicrobial resistance and nosocomial infections like CDI [37].

Several interventions have been implemented worldwide to contain CDI, including guidelines for infection control, education, intensification of hand hygiene promotion, use of additional contact precautions, and improvements in environmental control. Each of these interventions could have played a role in the CDI rate identified in our study. However, investigators suggest that a reduction in the use of high-risk antibiotics is the only apparent driver of the decrease in incidence of nosocomial CDI [24].

These findings suggest that the AMS program has focused its efforts in optimizing cephalosporins and clindamycin consumption as high-risk factors for CDI development, as these antibiotics present a strong relation for CDI development. ASP can help prevent CDI by optimizing antibiotic use, promote infection control measures, and increase public awareness about the disease. One of the main objectives of any ASP should be to make everyone a better steward, including patients and the public in general [38].

This study presents various strengths, key amongst them being the fact that this is the first study in the region that correlates CDI with hospital ward antibiotic prescription and the influence of specific breakpoints, such as Antimicrobial Stewardship Programs and the COVID-19 pandemic. Additionally, the inclusion of antifungals, antivirals, and antibiotics presents a sound base for a broader, more applicable analysis. The limitations of this study lie in the participation of patients for this study. Participation depended on available data from the Tertiary Care Hospitals. Furthermore, it was impossible to account for local antibiotic consumption and specific strain epidemiology like NAP1 or NAP9, yet this could pose a significant impact on nosocomial CDI. In addition, different healthcare priorities during the COVID-19 pandemic and potential diagnostic inconsistencies could have influenced our results. Finally, due to the nature of this ecological study, factors such as comorbidities, age, severity of infection, and the impact of said infection control measures could not be evaluated for analysis. Detailed knowledge about the risk of CDI after antibiotic exposure can aid clinicians in treating high-risk patients, improve antimicrobial stewardship, and, consequently, decrease the incidence of CDI.

Further studies about population level risk factors and CDI correlation, as well as studies to establish the impact of antibiotic susceptibility on the incidence of C. *difficile*, are needed in Latin America.

4. Materials and Methods

4.1. Study Design and Population

The ecological study conducted involved the examination of antimicrobial and PPIs consumption, correlated with nosocomial DCI incidence, coupled with time-series analysis. Patients with nosocomial CDI admitted to participating General Tertiary Care Hospitals from the Costa Rican Social Security (Hospital San Juan de Dios and Hospital Mexico) between January 2017 and December 2021 were included in the analysis. Participating hospitals are academic centers with the highest complexity level of health attention, covering 85% of the total Costa Rican population. Hospitals were selected based on data availability for antimicrobial and PPIs use, nosocomial CDI rates, and presence of AMS programs. Excluded from the data collection process were pediatric hospital wards, as well as any units that do not generate high discharge rates or occupied bed days (e.g., emergency services). The use of antibiotics in Costa Rican Social Security is regulated by an institutional medicines policy, based on the World Health Organization List of Essential Medicines. This study was approved by the Costa Rican Social Security Ethics Committee: CEC-CENTRAL-CCSS (Protocol #R022-SABI-00319).

4.2. Definitions and Surveillance

Nosocomial CDI was defined according to the Clinical Practice Guidelines for the Management of *Clostridioides difficile* Infection in Adults: 2021 Update by SHEA/IDSA [39] as: diarrhea (three or more unformed or liquid stools in less than 24 h) lasting 24 h or longer without any other cause, combined with a positive assay for toxigenic *C. difficile*, diagnosed via nucleic acid amplification test (NAATs) and enzyme immunoassays (EIA) as well as visualization of pseudo membranes by colonoscopy or histopathological diagnosis. A case was categorized as nosocomial if symptoms appeared 3 days or more after admission and up to 8 weeks after discharge. Definitions, laboratory assay to detect CDI and data collection methods did not change during the study period. The detection and categorization of CDI events were done retrospectively by personnel trained in infection prevention and control. Social Security tertiary care hospitals implemented AMS programs starting in January 2018. These programs include the surveillance of all antibiotic prescriptions, and any follow up of pre surgical antibiotic prophylaxis. ASP team gave prospective audits of antimicrobial prescriptions and provided real-time feedback to prescribing physicians.

4.3. Variables

Participating hospitals provided monthly nosocomial CDI rates per 100 bed days, according to the Infection Prevention and Control Units records, where this trend was evaluated. Furthermore, monthly data for 44 different antimicrobials available in the participating hospitals and PPIs consumption, calculated in DDD per 100 bed days, was obtained throughout the 60-month study period (January 2017 to December 2021) aided by the pharmaceutical software "Integrated Pharmacy System" (SIFA) version 3.0.5.3. DDD was chosen as the unit of measurement because is the recommended metric by the WHO, as well as reflecting a combination of dosage and duration of treatment [40]. WHO ATC-DDD 2023 index was employed for all 60 months. Before starting the study, the responsible pharmacists were trained in WHO ATC-DDD calculation to guarantee a homogeneous collection of data. Analysis was conducted individually for all 44 different antimicrobials and PPIs. Groups and families of antibiotics were classified according to the WHO AWaRe (Access, Watch and Reserve) classification.

4.4. Statistical Analysis

First, as a method of descriptive analysis, annual rates of nosocomial CDI and annual antimicrobial and PPIs consumption trends were described. Changes in antimicrobial consumption during the study period were evaluated using a linear regression test. The AAPC, with 95 percent confidence intervals, for different antimicrobials and PPIs was incorporated into the analysis to measure the average percentage change in antimicrobial consumption over time, thus combining the year-to-year fluctuations into a single value. The statistical analysis was conducted in two main stages: correlation analysis and interrupted time series analysis using Poisson's regression model. Correlation analysis was performed using Spearman's test to assess the monotonic association between the monthly antimicrobial and PPIs consumption with CDI incidence rate. This non-parametric test was chosen due to its robustness to non-normal distributions and potential non-linear relationships. The incidence of CDI and the antimicrobial and PPI consumption were paired without time intervals and with 1-month interval to determine the immediate and delayed effects of drug consumption on the incidence of CDI [41].

The second stage of the analysis included interrupted time-series methods to evaluate the impact of key temporal breakpoints on CDI incidence rate. A segmented Poisson's regression model was employed, given its appropriateness for analyzing count data with time-dependent changes. The interrupted time-series allows for the evaluation of immedi-

ate changes in level, coupled with long-term effects due to trend changes. The time series is divided (interrupted) into pre and post breakpoints [24].

Least square regression lines are fitted to each segment of the time series and together expressed by the following model:

$$Yt = \beta 0 + \beta 1 * time1 + \beta 2 * breakepoint1 + \beta 3 * timeafterbreakepoint1 + et$$

Yt represents any outcome at time point (t), which in this case is CDI incidence. Time is a continuous variable, measuring the number of months included in the entire time frame (60 months). $\beta 0$ estimated the baseline level at time point zero (y-axis intercept), $\beta 1$ estimates the baseline trend, $\beta 2$ estimates the level change between the pre and post breakpoint period, and $\beta 3$ estimates the change in trend after the breakpoint [42]. The sum of $\beta 1$ and $\beta 3$ estimates the post-breakpoint slope. The error et represents the variability of the model.

We distinguished three main breakpoints. Firstly, one year after the implementation of the ASP in the participating hospitals (January 2019). This was followed by the second and third periods, obtained during the COVID-19 pandemic, the first of which is set in March 2020, the month when the COVID-19 pandemic was officially declared; followed by September 2020, which is the month with the lowest rate of nosocomial CDI in the included time series. p values < 0.05 were considered statistically significant. Statistical analysis was performed using SAS Studio OnDemand for Academics (SAS Institute Inc., Cary, NC, USA) Version: 3.81 (Edition Enterprise).

5. Conclusions

The use of high-risk antibiotics, such as clindamycin, cephalosporins, and macrolides, were positively associated with CDI, despite some of these antibiotics showing consumption downtrends during our time series analysis. According to our analysis, specific events, such as the COVID-19 pandemic and the implementation of antimicrobial stewardship (ASP) programs, apparently did not impact CDI incidence. Further studies are needed in Latin America to improve our understanding of the role of individual risk factors and infection control measures with CDI.

Author Contributions: Conception and design: C.F.-B., S.G. and A.R.-E.; data collection C.F.-B., L.D.G.-Z., J.C.C. and M.R.-C.; the authors C.F.-B., S.G. and L.E.H.-S. had full access to raw data and verified the data. A.R.-E. and J.C.C. did the statistical analyses. All authors assisted in data interpretation. C.F.-B. and S.G. drafted the manuscript, which was revised by all authors. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Abbreviations

The following abbreviations are used in this manuscript:

PPIs	Proton Pump Inhibitors
CDI	*Clostridiodes difficile* infection
CDC	The United Stated Center of Disease Control and Prevention
GIPCR	Gastrointestinal tractPolymerase chain reaction
ASP	Antimicrobial Stewardship Program
DDD	Defined Daily Dose
WHO	World Health Organization
AWaRe	Access, Watch and Reserve
AAPC	Average Annual Percent Change
SIFA	Integrated Pharmacy System

Appendix A

Table A1. Change in hospital antimicrobial and proton pump inhibitors consumption around major breakpoints and their relationship with the incidence of nosocomial C. *difficile* in Tertiary Care Hospitals in Costa Rica.

Antimicrobials	January 2019			March 2020			September 2020		
	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint
AWaRe									
Access	0.0005 (−0.0095 to 0.0083) $p = 0.91$	−0.2573 (−6.047 to 5.3939) $p = 0.91$	−0.0353 (−0.3994 to 0.2945) $p = 0.82$	0.0008 (−0.0100 to 0.0089) $p = 0.86$	−0.8528 (−9.6531 to 5.0836) $p = 0.78$	0.0214 (−0.4704 to 0.5136) $p = 0.78$	0.0003 (−0.0097 to 0.0080) $p = 0.95$	−1.04 (−14.6290 to 4.6376) $p = 0.77$	0.0715 (−0.6724 to 1.0190) $p = 0.82$
Reserve	0.0036 (−0.0536 to 0.0363) $p = 0.85$	−0.3486 (−6.3406 to 5.4375) $p = 0.89$	−0.0378 (−0.3930 to 0.2941) $p = 0.81$	0.0050 (−0.0520 to 0.0500) $p = 0.83$	−0.9107 (−9.7930 to 4.8564) $p = 0.77$	0.0267 (−0.4590 to 0.5351) $p = 0.90$	0.0016 (−0.0525 to 0.0382) $p = 0.93$	−1.0131 (−14.6226 to 4.7297) $p = 0.78$	0.0708 (−0.6696 to 1.0243) $p = 0.83$
Watch	0.0003 (−0.0056 to 0.0041) $p = 0.89$	−0.2667 (−6.0762 to 5.3863) $p = 0.91$	−0.0328 (−0.3875 to 0.2964) $p = 0.83$	0.0006 (−0.0058 to 0.0054) $p = 0.82$	−0.9156 (−9.7837 to 4.7265) $p = 0.77$	0.0318 (−0.4546 to 0.5595) $p = 0.89$	0.0003 (−0.0058 to 0.0048) $p = 0.90$	−1.0178 (−14.6046 to 4.6610) $p = 0.78$	0.0779 (−0.6753 to 1.0312) $p = 0.81$
Antivirals									
Antivirals	0.0006 (−0.1114 to 0.0460) $p = 0.98$	−0.2011 (−5.6816 to 5.4144) $p = 0.93$	−0.0334 (−0.3876 to 0.2953) $p = 0.83$	0.0040 (−0.1130 to 0.0880) $p = 0.92$	−0.7728 (−10.1954 to 4.8132) $p = 0.82$	0.0143 (−0.4587 to 0.5467) $p = 0.94$	−0.0013 (−0.1134 to 0.0497) $p = 0.97$	−1.0623 (−14.6450 to 4.8109) $p = 0.77$	0.0697 (−0.6772 to 1.0225) $p = 0.83$
Acyclovir	−0.0023 (−0.3019 to 0.0650) $p = 0.97$	−0.2011 (−5.6816 to 5.4144) $p = 0.93$	−0.0334 (−0.3876 to 0.2953) $p = 0.83$	0.0048 (−0.3019 to 0.1549) $p = 0.94$	−0.7324 (−11.8190 to 4.8138) $p = 0.83$	0.0114 (−0.4600 to 0.6259) $p = 0.95$	0.0008 (−0.3176 to 0.0723) $p = 0.98$	−1.0550 (−14.6492 to 5.2825) $p = 0.77$	0.0677 (−0.6860 to 1.0219) $p = 0.84$
Ganciclovir	0.0096 (−0.1965 to 0.1369) $p = 0.89$	−0.2743 (−6.0194 to 5.4043) $p = 0.91$	−0.0360 (−0.3972 to 0.2945) $p = 0.82$	0.0104 (−0.1973 to 0.1552) $p = 0.89$	−0.7208 (−9.4310 to 4.6557) $p = 0.80$	0.0137 (−0.4527 to 0.4991) $p = 0.94$	0.0067 (−0.1974 to 0.1386) $p = 0.93$	−1.0444 (−14.6218 to 4.6417) $p = 0.77$	0.0715 (−0.6713 to 1.0187) $p = 0.82$
Valganciclovir	−0.0077 (−0.3437 to 0.1892) $p = 0.95$	−0.2069 (−5.4417 to 5.3905) $p = 0.93$	−0.0327 (−0.3883 to 0.2973) $p = 0.83$	−0.0001 (−0.0010 to 0.0007) $p = 0.75$	−0.0243 (−0.0427 to −0.0059) $p = 0.008$	0.0000 (−0.0012 to 0.0013) $p = 0.93$	−0.0050 (−0.3455 to 0.2012) $p = 0.96$	−1.0311 (−14.6376 to 4.6705) $p = 0.78$	0.0649 (−0.6696 to 1.0143) $p = 0.84$
Antifungals									
Antifungals	0.0000 (−0.0244 to 0.0194) $p = 0.99$	−0.1941 (−5.9013 to 5.4732) $p = 0.93$	−0.0332 (−0.3911 to 0.2966) $p = 0.84$	0.0006 (−0.0258 to 0.0217) $p = 0.96$	−0.6977 (−10.0415 to 4.9719) $p = 0.83$	0.0095 (−0.4546 to 0.5455) $p = 0.96$	0.0005 (−0.0261 to 0.0189) $p = 0.95$	−1.1049 (−14.7746 to 5.2328) $p = 0.77$	0.0747 (−0.6901 to 1.0552) $p = 0.83$
Anidulafungin	0.0024 (0.0660 to 0.0399) $p = 0.92$	−0.2628 (−6.0873 to 5.4242) $p = 91$	−0.0342 (−0.3878 to 0.2951) $p = 0.82$	0.0047 (−0.065 to 0.0610) $p = 0.87$	−0.8567 (−9.9297 to 4.7077) $p = 0.79$	0.0231 (−0.4529 to 0.5497) $p = 0.92$	0.0023 (−0.0658 to 0.0431) $p = 0.92$	−1.0605 (−14.6330 to 4.6767) $p = 0.77$	0.0753 (−0.6761 to 1.0301) $p = 0.82$
Caspofungin	0.0128 (−0.4322 to 0.2290) $p = 0.92$	−0.2431 (−5.9853 to 5.4011) $p = 0.92$	−0.0325 (−0.3867 to 0.2993) $p = 0.83$	0.0354 (−0.4600 to 0.3618) $p = 0.84$	−0.9395 (−9.7380 to 5.1209) $p = 0.77$	0.0322 (−0.4875 to 0.5582) $p = 0.89$	0.0090 (−0.4552 to 0.2759) $p = 0.95$	−1.0202 (−14.6645 to 4.6453) $p = 0.78$	0.0708 (−0.6870 to 1.0178) $p = 0.83$

Table A1. *Cont.*

Antimicrobials	January 2019			March 2020			September 2020		
	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint
Fluconazole	−0.0009 (−0.0366 to 0.0263) $p = 0.95$	−0.1885 (−5.4794 to 5.4019) $p = 0.93$	−0.0315 (−0.3888 to 0.2982) $p = 0.84$	−0.0004 (−0.0373 to 0.0258) $p = 0.97$	−0.5950 (−9.7314 to 4.6668) $p = 0.84$	0.0039 (−0.4515 to 0.5104) $p = 0.98$	0.0005 (−0.0390 to 0.0256) $p = 0.97$	−1.1039 (−15.0177 to 5.3354) $p = 0.78$	0.0714 (−0.6781 to 1.0636) $p = 0.84$
Itraconazole	0.0054 (−0.3994 to 0.01881) $p = 0.96$	−0.2359 (−5.8501 to 5.5260) $p = 0.92$	−0.0338 (−0.3870 to 0.2952) $p = 0.83$	−0.0015 (−0.3965 to 0.1625) $p = 0.98$	−0.6241 (−9.3305 to 4.5433) $p = 0.82$	0.0047 (−0.4503 to 0.4819) $p = 0.98$	0.0037 (−0.3926 to 0.1705) $p = 0.97$	−1.0524 (−14.6372 to 4.6629) $p = 0.77$	0.0670 (−0.6702 to 1.0140) $p = 0.83$
Ketoconazole	0.0467 (−3.1821 to 1.4258) $p = 0.96$	−0.1981 (−5.4691 to 5.3960) $p = 0.93$	−0.0332 (−0.3886 to 0.2957) $p = 0.83$	0.1021 (−3.1558 to 1.8541) $p = 0.91$	−0.6988 (−9.4203 to 4.6571) $p = 0.81$	0.0097 (−0.4637 to 0.4878 $p = 0.96$	0.0561 (−3.1593 to 1.5261) $p = 0.95$	−1.0519 (−14.6398 to 4.6383) $p = 0.77$	0.0683 (−0.6694 to 1.0169) $p = 0.83$
Voriconazole	−0.0024 (−0.1727 to 0.0921) $p = 0.89$	−0.1538 (−6.1370 to 5.6403) $p = 0.95$	−0.0325 (−0.3878 to 0.2968) $p = 0.83$	−0.0002 (−0.1794 to 0.1184) $p = 0.99$	−0.6175 (−9.4550 to 5.4526) $p = 0.84$	0.0045 (−0.5070 to 0.4995) $p = 0.98$	−0.0052 (−0.1707 to 0.0936) $p = 0.92$	−1.0608 (−14.8026 to 4.7114) $p = 0.77$	0.0594 (−0.6986 to 1.0148) $p = 0.85$
Antibiotics	0.0002 (−0.0034 to 0.0027) $p = 0.89$	−0.2759 (−6.1584 to 5.3900) $p = 0.91$	−0.0341 (−0.3911 to 0.2951) $p = 0.83$	0.0004 (−0.0036 to 0.0035) $p = 0.82$	−0.9326 (−9.8016 to 4.8605) $p = 0.77$	0.0309 (−0.4599 to 0.5469) $p = 0.89$	0.0002 (−0.0035 to 0.0029) $p = 0.91$	−1.0244 (−14.6110 to 4.6520) $p = 0.78$	0.0759 (−0.6741 to 1.0271) $p = 0.82$
Antibiotics	0.0030 (−0.0153 to 0.0305) $p = 0.87$	−0.2689 (−5.8877 to 5.3796) $p = 0.91$	−0.0376 (−0.4007 to 0.2946) $p = 0.81$	0.0046 (−0.0571 to 0.0477) $p = 84$	−0.8860 (−10.0507 to 4.7371) $p = 0.78$	0.0196 (−0.4505 to 0.5315) $p = 0.92$	0.0016 (−0.0569 to 0.0326) $p = 0.93$	−1.0418 (−14.6712 to 4.6642) $p = 0.77$	0.0701 (−0.6691 to 1.0328) $p = 0.83$
Aminoglycosides	0.0029 (−0.0572 to 0.0312) $p = 0.88$	−0.2676 (−5.9186 to 5.3816) $p = 0.91$	−0.0376 (−0.4007 to 0.2946) $p = 0.81$	0.0046 (−0.0571 to 0.0477) $p = 0.84$	−0.8860 (−10.0507 to 4.7371) $p = 0.78$	0.0196 (−0.4505 to 0.5315) $p = 0.92$	0.0016 (−0.0569 to 0.0326) $p = 0.93$	−1.0418 (−14.6712 to 4.6642) $p = 0.77$	0.0701 (−0.6691 to 1.0328) $p = 0.83$
Amikacin	0.0075 (−0.2371 to 0.1844) $p = 0.94$	−0.1954 (−5.4266 to 5.3899) $p = 0.93$	−0.0333 (−0.3861 to 0.2952) $p = 0.83$	0.0067 (−0.2371 to 0.1840) $p = 0.94$	−0.6146 (−9.3075 to 4.5515) $p = 0.83$	0.0042 (−0.4530 to 0.4816) $p = 0.98$	0.0091 (−0.2366 to 0.1872) $p = 0.92$	−1.0626 (−14.6321 to 4.6383) $p = 0.77$	0.0664 (−0.6688 to 1.0128) $p = 0.83$
Gentamicin	0.0009 (−0.0216 to 0.0139) $p = 0.91$	−0.2563 (−6.0278 to 5.3985) $p = 0.91$	−0.033 (−0.3885 to 0.2970) $p = 0.83$	0.0021 (−0.0228 to 0.0227) $p = 0.83$	−0.9612 (−10.0725 to 4.7836) $p = 0.77$	0.0331 (−0.4581 to 0.5886) $p = 0.89$	0.0009 (−0.0226 to 0.0164) $p = 0.91$	−1.0445 (−14.6088 to 4.6325) $p = 0.71$	0.0786 (−0.6772 to 1.0280) $p = 0.81$
Carbapenems	0.0070 (−0.5594 to 0.3352) $p = 0.97$	−0.1792 (−5.5257 to 5.4647) $p = 0.94$	−0.0346 (−0.4447 to 0.3229) $p = 0.84$	0.0023 (−0.5844 to 0.3442) $p = 0.99$	−0.6225 (−9.3228 to 4.5459) $p = 0.82$	0.0036 (−0.5077 to 0.5251) $p = 0.98$	−0.0015 (−0.6073 to 0.3836) $p = 0.99$	−1.0427 (−14.7872 to 4.7854) $p = 0.78$	0.0671 (−0.6886 to 1.0260) $p = 0.84$
Aztreonam	0.0038 (−0.0700 to 0.0413) $p = 0.88$	−0.2265 (−5.6727 to 5.3886) $p = 0.92$	−0.0311 (−0.3864 to 0.3051) $p = 0.84$	0.0082 (−0.0701 to 0.0716) $p = 0.79$	−0.9865 (−10.1565 to 4.6538) $p = 0.76$	0.0323 (−0.4541 to 0.5656) $p = 0.88$	0.0030 (−0.0714 to 0.0495) $p = 0.91$	−0.9810 (−14.6279 to 5.0792) $p = 0.79$	0.0716 (−0.6685 to 1.0404) $p = 0.82$
Ertapenem	0.0258 (−1.1997 to 0.3109) $p = 0.91$	−0.2397 (−5.7173 to 5.3882) $p = 0.92$	−0.0329 (−0.3866 to 0.2970) $p = 0.83$	0.0444 (−1.1975 to 0.5158) $p = 0.87$	−0.7682 (−9.5684 to 4.6221) $p = 0.80$	0.0164 (−0.4594 to 0.5074) $p = 0.93$	0.0124 (−1.2308 to 0.3410) $p = 0.96$	−1.0225 (−14.6374 to 4.8281) $p = 0.78$	0.0670 (−0.6695 to 1.0153) $p = 0.83$
Imipenem Cilastatin	0.0009 (−0.0256 to 0.0196) $p = 0.93$	−0.2458 (−6.0018 to 5.4393) $p = 0.92$	−0.0332 (−0.3879 to 0.2956) $p = 0.83$	0.0019 (−0.0273 to 0.0271) $p = 0.88$	−0.8254 (−9.7619 to 4.7843) $p = 0.79$	0.0233 (−0.4603 to 0.5497) $p = 0.92$	0.0011 (−0.0274 to 0.0221) $p = 0.92$	−1.0670 (−14.6158 to 4.6710) $p = 0.77$	0.0789 (−0.6875 to 1.0223) $p = 0.82$
Meropenem	0.0010 (−0.0134 to 0.0118) $p = 0.87$	−0.2691 (−5.9553 to 5.3721) $p = 0.91$	−0.0328 (−0.3895 to 0.2955) $p = 0.83$	0.0014 (−0.0319 to 0.0126) $p = 0.82$	−0.8180 (−9.5002 to 4.7667) $p = 0.78$	0.0254 (−0.4626 to 0.5167) $p = 0.90$	0.0008 (−0.4626 to 0.5167) $p = 0.90$	−0.9912 (−14.5968 to 4.7190) $p = 79$	0.0748 (−0.6701 to 1.0233) $p = 82$
Cephalosporins	0.0007 (−0.0560 to 0.0335) $p = 0.97$	−0.1878 (−5.4345 to 5.4309) $p = 0.93$	−0.0321 (−0.3861 to 0.3065) $p = 0.84$	0.0013 (−0.0530 to 0.0434) $p = 0.95$	−0.6159 (−9.3160 to 4.5364) $p = 0.82$	0.0062 (−0.4606 to 0.4821) $p = 0.97$	0.0006 (−0.0510 to 0.0361) $p = 0.97$	−1.0322 (−14.6544 to 4.9047) $p = 0.78$	0.0659 (−0.6708 to 1.0142) $p = 0.83$
Cephalexin	0.0031 (−0.1079 to 0.0474) $p = 0.92$	−0.2308 (−5.7018 to 5.3955) $p = 0.92$	−0.0336 (−0.3886 to 0.2946) $p = 0.83$	0.0040 (−0.1089 to 0.0676) $p = 0.91$	−0.6909 (−9.3205 to 4.7441) $p = 0.81$	0.0131 (−0.4898 to 0.4908) $p = 0.95$	0.0003 (−0.1140 to 0.0504) $p = 0.99$	−1.0408 (−14.7199 to 4.9238) $p = 0.78$	0.0664 (−0.6700 to 1.0145) $p = 0.83$
Cephalothin	0.0009 (−0.0198 to 0.0162) $p = 0.91$	−0.2547 (−5.8497 to 5.3967) $p = 0.91$	−0.0323 (−0.3858 to 0.2955) $p = 0.83$	0.0014 (−0.0197 to 0.0177) $p = 0.87$	−0.7515 (−9.5098 to 4.5944) $p = 0.80$	0.0188 (−0.4505 to 0.5188) $p = 0.93$	0.0008 (−0.0197 to 0.0165) $p = 0.92$	−1.0258 (−14.6159 to 4.6675) $p = 0.78$	0.0739 (−0.6724 to 1.0241) $p = 0.82$
Cefotaxime	0.0149 (−0.9332 to 0.4016) $p = 0.95$	−0.1759 (−5.4946 to 5.4344) $p = 0.94$	−0.0364 (−0.4067 to 0.3120) $p = 0.82$	0.0194 (−1.0239 to 0.4358) $p = 0.94$	−0.6469 (−9.4814 to 4.5897) $p = 0.82$	0.0001 (−0.4531 to 0.5139) $p = 0.99$	0.0083 (−1.0845 to 0.5016) $p = 0.97$	−1.0599 (−14.8155 to 4.6740) $p = 0.72$	0.0642 (−0.6878 to 1.0166) $p = 0.84$
Ceftazidime/ Avibactam	0.0003 (−0.6986 to 0.1869) $p = 0.99$	−0.1957 (−5.5017 to 5.4136) $p = 0.93$	−0.0332 (−0.3862 to 0.2957) $p = 0.83$	−0.0001 (−0.7155 to 0.2331) $p = 0.99$	−0.6215 (−9.3457 to 4.6171) $p = 0.82$	0.0048 (−0.4815 to 0.4819) $p = 0.98$	−0.0103 (−0.7190 to 0.1975) $p = 0.94$	−1.1012 (−14.7423 to 4.9203) $p = 0.77$	0.0676 (−0.6696 to 1.0160) $p = 0.83$
Ceftaroline									

Table A1. *Cont.*

Antimicrobials	January 2019			March 2020			September 2020		
	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint
Ceftazidime	0.0211 (−0.0444 to 0.0426) $p = 0.84$	−0.2729 (−5.8625 to 5.3586) $p = 0.91$	−0.0407 (−0.4105 to 0.2951) $p = 0.80$	0.0050 (−0.0441 to 0.04711) $p = 0.81$	−0.8657 (−9.6879 to 4.6134) $p = 0.77$	0.0166 (−0.4482 to 0.5021) $p = 0.93$	0.0032 (−0.0439 to 0.0427) $p = 0.87$	−1.0732 (−14.6659 to 4.7119) $p = 0.77$	0.0747 (−0.6697 to 1.0276) $p = 0.81$
Ceftolozane/ Tazobactam	0.0265 (−1.1830 to 0.3649) $p = 0.92$	−0.2028 (−5.4502 to 5.3868) $p = 0.93$	−0.0298 (−0.3877 to 0.3054) $p = 0.85$	0.0454 (−1.1748 to 0.5019) $p = 0.87$	−0.6897 (−9.3541 to 4.5374) $p = 0.81$	0.0144 (−0.4564 to 0.5001) $p = 0.94$	0.0240 (−1.1725 to 0.4229) $p = 0.93$	−1.0012 (−14.6202 to 4.9676) $p = 0.78$	0.0678 (−0.6689 to 1.0160) $p = 0.83$
Ceftriaxone	−0.5503 (−50.6005 to 10.2708) $p = 0.95$	−0.1941 (−5.4509 to 5.4293) $p = 0.93$	−0.0336 (−0.4004 to 0.2975) $p = 0.83$	−0.5907 (−50.4747 to 10.1571) $p = 0.94$	−0.6290 (−9.3234 to 4.5381) $p = 0.82$	0.0052 (−0.4501 to 0.4824) $p = 0.97$	−0.5627 (−50.6230 to 10.1139) $p = 0.94$	−1.0503 (−14.6426 to 4.6402) $p = 0.77$	0.0668 (−0.6694 to 1.0147) $p = 0.83$
Macrolides	0.0025 (−0.1079 to 0.0630) $p = 0.94$	−0.1506 (−5.4861 to 5.5395) $p = 0.95$	−0.0293 (−0.3901 to 0.3156) $p = 0.86$	0.0059 (−0.0994 to 0.0593) $p = 0.87$	−0.6899 (−9.3056 to 4.6110) $p = 0.81$	0.0137 (−0.4757 to 0.4854) $p = 0.94$	0.0024 (−0.1071 to 0.0589) $p = 79$	−0.9884 (−14.6888 to 5.0126) $p = 0.83$	0.0658 (−0.6695 to 1.0136) $p = 0.83$
Azithromycin	−0.0027 (−0.4889 to 0.1127) $p = 0.98$	−0.1795 (−5.6446 to 5.5019) $p = 0.94$	−0.0335 (−0.3865 to 0.2954) $p = 0.83$	−0.0005 (−0.4987 to 0.1943) $p = 0.99$	−0.6192 (−9.3150 to 4.6934) $p = 0.83$	0.0046 (−0.4864 to 0.4844) $p = 0.98$	−0.0082 (−0.5044 to 0.1202) $p = 0.94$	−1.0944 (−14.7725 to 4.6475) $p = 0.77$	0.0662 (−0.6857 to 1.0172) $p = 0.83$
Clarithromycin	0.0037 (−0.1169 to 0.0762) $p = 0.93$	−0.1074 (−5.5663 to 5.8119) $p = 0.96$	−0.0278 (−0.3897 to 0.3210) $p = 0.87$	0.0063 (−0.1039 to 0.0599) $p = 0.86$	−0.6569 (−9.3037 to 4.5210) $p = 0.81$	0.0104 (−0.4521 to 0.4839) $p = 0.95$	0.0040 (−0.1103 to 0.0598) $p = 0.92$	−0.9751 (−14.6237 to 4.9818) $p = 0.79$	0.0653 (−0.6699 to 1.0125) $p = 0.83$
Penicillins	0.0006 (−0.0162 to 0.0126) $p = 0.93$	−0.2502 (−5.9861 to 5.4333) $p = 0.91$	−0.0351 (−0.3935 to 0.2949) $p = 0.82$	0.0011 (−0.0167 to 0.0155) $p = 0.88$	−0.8206 (−9.7186 to 5.0138) $p = 0.79$	0.0176 (−0.4630 to 0.5114) $p = 0.93$	0.0002 (−0.0164 to 0.0125) $p = 0.98$	−1.0438 (−14.6543 to 4.6551) $p = 0.77$	0.0679 (−0.6701 to 1.0163) $p = 0.83$
Amoxicillin	0.0037 (−0.2388 to 0.2461) $p = 0.97$	−0.1863 (−4.8462 to 4.4736) $p = 0.93$	−0.0332 (−0.3431 to 0.2767) $p = 0.83$	0.0091 (−0.3166 to 0.2183) $p = 0.94$	−0.6600 (−9.3043 to 4.6607) $p = 0.82$	0.0072 (−0.4579 to 0.4826) $p = 0.97$	−0.0017 (−0.3143 to 0.2078) $p = 0.98$	−1.0558 (−14.9566 to 4.6997) $p = 0.77$	0.0666 (−0.6712 to 1.0477) $p = 0.83$
Ampicillin Sulbactam	−0.0025 (−0.4620 to 0.0389) $p = 0.95$	−0.1884 (−5.4420 to 5.4000) $p = 0.93$	−0.0335 (−0.3864 to 0.2948) $p = 0.83$	0.0002 (−0.4574 to 0.0810) $p = 0.99$	−0.6265 (−9.8086 to 4.7564) $p = 0.83$	0.0052 (−0.4822 to 0.4979) $p = 0.98$	−0.0041 (−0.4654 to 0.0420) $p = 0.93$	−1.0840 (−14.6877 to 4.6890) $p = 0.77$	0.0664 (−0.6703 to 1.0143) $p = 0.83$
Ampicillin	0.0041 (−0.1093 to 0.0801) $p = 0.92$	−0.2622 (−6.1881 to 5.4273) $p = 0.91$	−0.0338 (−0.3886 to 0.2953) $p = 0.83$	0.0054 (−0.1098 to 0.0947) $p = 0.91$	−0.7225 (−9.4998 to 4.6340) $p = 0.81$	0.0152 (−0.4531 to 0.5201) $p = 0.94$	0.0026 (−0.1101 to 0.0815) $p = 0.95$	−1.0392 (−14.6312 to 4.6463) $p = 0.77$	0.0711 (−0.6730 to 1.0222) $p = 0.83$
Oxacillin	0.0005 (−0.0245 to 0.01777) $p = 0.95$	−0.2183 (−5.6636 to 5.4175) $p = 0.92$	−0.0353 (−0.3995 to 0.2992) $p = 0.82$	0.0008 (−0.0243 to 0.0196) $p = 0.93$	−0.7108 (−9.7012 to 4.7101) $p = 0.81$	0.0081 (−0.4499 to 0.4958) $p = 0.96$	0.0002 (−0.0242 to 0.0166) $p = 0.98$	−1.0566 (−14.6511 to 4.6507) $p = 0.77$	0.0678 (−0.6704 to 1.0189) $p = 0.83$
Penicillin sodium	0.0012 (−0.0829 to 0.0621) $p = 0.97$	−0.1993 (−5.4415 to 5.3934) $p = 0.93$	−0.0338 (−0.3897 to 0.2952) $p = 0.83$	0.0028 (−0.0822 to 0.0707) $p = 0.93$	−0.6774 (−9.3499 to 4.6593) $p = 0.81$	0.0060 (−0.4513 to 0.4818) $p = 0.97$	−0.0001 (−0.0832 to 0.0577) $p = 0.99$	−1.0477 (−14.7006 to 4.6405) $p = 0.77$	0.0664 (−0.6700 to 1.0225) $p = 0.83$
Penicillin benzathine	−0.4872 (−22.9761 to 7.6030) $p = 0.94$	−0.2071 (−5.4255 to 5.3933) $p = 0.92$	−0.0315 (−0.3889 to 0.2973) $p = 0.84$	−0.1632 (−22.9182 to 10.3866) $p = 0.98$	−0.5970 (−9.4315 to 4.8454) $p = 0.84$	0.0039 (−0.4656 to 0.4841) $p = 0.98$	−0.3589 (−22.3922 to 8.0027) $p = 0.95$	−1.0227 (−14.6305 to 4.8773) $p = 0.78$	0.0639 (−0.6752 to 1.0138) $p = 0.84$
Piperacillin/ Tazobactam	0.0042 (−0.0788 to 0.0506) $p = 0.88$	−0.3372 (−6.3895 to 5.4647) $p = 0.89$	−0.0281 (−0.3866 to 0.3119) $p = 0.86$	0.0060 (−0.0821 to 0.0642) $p = 0.85$	−0.7485 (−9.3739 to 4.6351) $p = 0.79$	0.0309 (−0.5079 to 0.5642) $p = 0.90$	0.0025 (−0.0910 to 0.0654) $p = 0.94$	−0.9480 (−14.7637 to 5.2800) $p = 0.81$	0.0721 (−0.6824 to 1.0233) $p = 0.82$
Fluoroquinolones	0.0034 (−0.0490 to 0.0390) $p = 0.87$	−0.2477 (−5.9084 to 5.3761) $p = 0.91$	−0.0398 (−0.4155 to 0.2943) $p = 0.80$	0.0057 (−0.0493 to 0.0541) $p = 0.81$	−0.9987 (−10.1611 to 4.7569) $p = 0.76$	0.0238 (−0.4465 to 0.5338) $p = 0.91$	0.0026 (−0.0490 to 0.0404) $p = 0.89$	−1.0794 (−14.7321 to 4.6991) $p = 0.77$	0.0758 (−0.6692 to 1.0445) $p = 0.81$
Ciprofloxacin	0.0060 (−0.1373 to 0.0595) $p = 0.88$	−0.1623 (−5.4154 to 5.4641) $p = 0.94$	−0.0321 (−0.3885 to 0.2992) $p = 0.83$	0.0098 (−0.1267 to 0.0892) $p = 0.82$	−0.7748 (−9.4248 to 4.5496) $p = 0.79$	0.0130 (−0.4516 to 0.4825) $p = 0.94$	0.0048 (−0.1301 to 0.0657) $p = 0.90$	−0.9960 (−14.6064 to 4.7720) $p = 0.78$	0.0647 (−0.6688 to 1.0122) $p = 0.84$
Levofloxacin	0.0028 (−0.0610 to 0.0428) $p = 0.90$	−0.2540 (−5.9819 to 5.3888) $p = 0.91$	−0.0392 (−0.4158 to 0.2993) $p = 0.81$	0.0040 (−0.0602 to 0.0541) $p = 0.88$	−0.8302 (−10.0717 to 4.7572) $p = 0.79$	0.0150 (−0.4489 to 0.5364) $p = 0.94$	0.0021 (−0.0612 to 0.0433) $p = 0.92$	−1.0954 (−14.7874 to 4.9179) $p = 0.77$	0.0747 (−0.6721 to 1.0527) $p = 0.82$
Tetracyclines	−0.0007 (−0.2322 to 0.1326) $p = 0.99$	−0.1960 (−5.4300 to 5.4178) $p = 0.93$	−0.0333 (−0.3864 to 0.2978) $p = 0.83$	−0.0020 (−0.2359 to 0.1328) $p = 0.98$	−0.6262 (−9.3176 to 4.5610) $p = 0.82$	0.0047 (−0.4505 to 0.4826) $p = 0.98$	0.0055 (−0.2223 to 0.1461) $p = 0.94$	−1.0864 (−14.6932 to 4.7053) $p = 0.77$	0.0695 (−0.6700 to 1.0184) $p = 0.83$
Doxycycline	−0.0034 (−0.2487 to 0.1328) $p = 0.97$	−0.1976 (−5.4292 to 5.4082) $p = 0.93$	−0.0334 (−0.3870 to 0.2976) $p = 0.83$	−0.0037 (−0.2544 to 0.1350) $p = 0.96$	−0.6251 (−9.3182 to 4.5419) $p = 0.82$	0.0046 (−0.4509 to 0.4818) $p = 0.98$	0.0024 (−0.2418 to 0.1495) $p = 0.97$	−1.0615 (−14.6577 to 4.7202) $p = 0.77$	0.0673 (−0.6723 to 1.0148) $p = 0.83$

Table A1. *Cont.*

Antimicrobials	January 2019			March 2020			September 2020		
	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint	Hospital Onset CDI per Period	Breakpoint	Change in Trend at Breakpoint
Tigecycline	0.0230 (−1.0965 to 0.3221) $p = 0.92$	−0.1823 (−5.4245 to 5.3987) $p = 0.93$	−0.0326 (−0.3876 to 0.2947) $p = 0.83$	0.0124 (−1.1002 to 0.3734) $p = 0.96$	−0.6016 (−9.3097 to 4.7612) $p = 0.83$	0.0052 (−0.4506 to 0.4875) $p = 0.97$	0.0279 (−1.0802 to 0.3436) $p = 0.90$	−1.0728 (−14.7052 to 4.6447) $p = 0.77$	0.0705 (−0.6716 to 1.0265) $p = 0.82$
Clindamycin	0.0063 (−0.1528 to 0.1220) $p = 0.92$	−0.2619 (−5.7069 to 5.4470) $p = 0.91$	−0.0369 (−0.4103 to 0.2983) $p = 0.82$	0.0025 (−0.1520 to 0.0981) $p = 0.96$	−0.6220 (−9.3111 to 4.5506) $p = 0.82$	0.0052 (−0.4509 to 0.4818) $p = 0.97$	0.0014 (−0.1530 to 0.0958) $p = 0.98$	−1.0422 (−14.6440 to 4.6435) $p = 0.77$	0.0661 (−0.6706 to 1.0144) $p = 0.83$
Linezolid	0.0031 (−0.0378 to 0.0439) $p = 0.88$	−0.3288 (−5.3332 to 4.6755) $p = 0.89$	−0.0352 (−0.3460 to 0.2756) $p = 0.82$	0.0051 (−0.0585 to 0.0546) $p = 0.83$	−0.9266 (−10.0027 to 4.7608) $p = 0.77$	0.0290 (−0.4552 to 0.5611) $p = 0.90$	0.0014 (−0.0595 to 0.0377) $p = 0.94$	−1.0183 (−14.6307 to 4.7429) $p = 0.78$	0.0708 (−0.6724 to 1.0240) $p = 0.83$
Metronidazole	0.0086 (−0.0837 to 0.0698) $p = 0.81$	−0.1923 (−5.4642 to 5.4194) $p = 0.93$	−0.0288 (−0.3845 to 0.3065) $p = 0.85$	0.0081 (−0.0829 to 0.0762) $p = 0.82$	−0.5768 (−9.2517 to 4.5816) $p = 0.84$	0.0155 (−0.4470 to 0.4857) $p = 0.95$	0.0082 (−0.0838 to 0.0729) $p = 0.82$	−0.9575 (−14.5238 to 4.9156) $p = 0.79$	0.0690 (−0.6719 to 1.0088) $p = 0.83$
Nitrofurantoin	−0.0039 (−0.5718 to 0.3932) $p = 0.98$	−0.1986 (−5.4531 to 5.3944) $p = 0.93$	−0.0339 (−0.4006 to 0.2956) $p = 0.83$	0.0017 (−0.5305 to 0.3833) $p = 0.99$	−0.6220 (−9.3190 to 4.5470) $p = 0.82$	0.0052 (−0.4598 to 0.4828) $p = 0.97$	0.0013 (−0.5313 to 0.3821) $p = 0.99$	−1.0457 (−14.7024 to 4.6427) $p = 0.77$	0.0665 (−0.6754 to 1.0156) $p = 0.83$
Polymyxin B	0.0084 (−0.3206 to 0.1544) $p = 0.93$	−0.2024 (−5.4773 to 5.3986) $p = 0.93$	−0.0349 (−0.3883 to 0.2947) $p = 0.82$	0.0032 (−0.3188 to 0.1621) $p = 0.97$	−0.6172 (−9.3126 to 4.5545) $p = 0.83$	0.0050 (−0.4504 to 0.4834) $p = 0.97$	0.0064 (−0.3186 to 0.1568) $p = 0.95$	−1.0568 (−14.6559 to 4.6370) $p = 0.77$	0.0676 (−0.6704 to 1.0171) $p = 0.83$
Rifampicin	−0.0140 (−0.4889 to 0.2730) $p = 0.93$	−0.2180 (−5.4304 to 5.4267) $p = 0.92$	−0.0356 (−0.3864 to 0.3053) $p = 0.82$	0.0067 (−0.4801 to 0.3678) $p = 0.97$	−0.6649 (−9.5070 to 5.0639) $p = 0.83$	0.0079 (−0.5046 to 0.4958) $p = 0.97$	−0.0157 (−0.4863 to 0.3025) $p = 0.93$	−1.0939 (−14.7116 to 4.6694) $p = 0.77$	0.0630 (−0.6846 to 1.0128) $p = 0.84$
Sulfamethoxazole and Trimethoprim	0.0001 (−0.0382 to 0.0290) $p = 0.99$	−0.1951 (−5.8733 to 5.4199) $p = 0.93$	−0.0332 (−0.3880 to 0.2968) $p = 0.83$	0.0010 (−0.0386 to 0.0384) $p = 0.95$	−0.6968 (−9.6623 to 4.7622) $p = 0.82$	0.0113 (−0.4554 to 0.5422) $p = 0.96$	0.0006 (−0.0386 to 0.0353) $p = 0.97$	−1.0616 (−14.6365 to 4.9949) $p = 0.77$	0.0714 (−0.7402 to 1.0268) $p = 0.83$
Vancomycin	0.0009 (−0.0233 to 0.0157) $p = 0.91$	−0.2509 (−5.9697 to 5.4052) $p = 0.91$	−0.0326 (−0.3859 to 0.2958) $p = 0.83$	0.0015 (−0.0228 to 0.0174) $p = 0.87$	−0.7914 (−9.5916 to 4.7207) $p = 0.79$	0.0218 (−0.4588 to 0.5312) $p = 0.92$	0.0007 (−0.0228 to 0.0166) $p = 0.93$	−1.0279 (−14.6215 to 4.6666) $p = 0.78$	0.0738 (−0.6754 to 1.0275) $p = 0.82$
Proton pump inhibitors	0.0004 (−0.0059 to 0.0050) $p = 0.86$	−0.3358 (−6.2083 to 5.4348) $p = 0.89$	−0.0260 (−0.3920 to 0.3111) $p = 0.87$	0.0005 (−0.0061 to 0.0048) $p = 0.84$	−0.6949 (−9.3610 to 4.5548) $p = 0.80$	0.0263 (−0.4669 to 0.5397) $p = 0.90$	0.0004 (−0.0062 to 0.0048) $p = 87$	−0.9483 (−14.5743 to 5.0486) $p = 0.80$	0.0808 (−0.6678 to 1.0348) $p = 0.81$

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5

Discussion

To the extent of our knowledge, this study is one of the first multicentric investigations in the Latin America region to document the association between antimicrobial and PPI consumption, clinical risk factors and HA-CDI through a population-based case-control study with PSM and an ecological analysis. The aim of this research was to inform ASPs regarding decision making related to antimicrobial selection and other clinical risk factors such as comorbidities, ICU admission and hospital length of stay that may contribute to increased HA-CDI incidence.

CDI is an increasing public health problem worldwide. HA-CDI incidence rates vary by geography and over time but have generally increased in the past 30 years (46). However, data on CDI incidence in hospitals in middle and low-income countries in Latin America, Asia and Easter Europe is limited. For this reason, data on microbiology, infection rates and clinical characteristics are needed to better understand local and regional behavior of these infections, thus, infection control policies can be implemented according to local resources and needs (145).

In our study, the mean incidence of CDI between 2017 and 20121 was 0.03 infections per 100 bed days, a lower incidence rate than reported in other regions. As an example, a multicentric study in Germany evaluated nosocomial CDI from 2016 to 2020, reporting 0.30 cases per 100 patients in 2016 and 0.26 patients in 2020 (141). In the Latin America region, a multicentric study conducted in Mexico from 2011 to 2015, reported 0.28 pr 1000 patients-days as average rate of CDI, this reported rate was also lower than previous studies in Poland reporting 0.6, 0.8 and 0.9 cases per 1000 patient days in a 2011 to 2013 series. However, given there are no precise data of the incidence rate in Latin America, no conclusion can be drawn about the regional incidence rate (145).

It is important to point out that this study was conducted during COVID-19 pandemic. The pandemic impact on CDI incidence has shown mixed findings according to published studies, evidence suggests that hospitalized COVID-19 patients have a higher risk for CDI, however, no significant change or a decline in CDI cases during the early pandemic years is identified, as shown in this investigation (143). Several factors may have influenced this trend, including changes in patient management and the suspension of elective surgeries and routine medical care for non-COVID-19 conditions due to strict lockdown measures implemented in Costa Rica. Additionally, enhanced infection prevention strategies, such as visitor restrictions, improved hand hygiene, increased use of personal protective

equipment, and the isolation of COVID-19 patients in designed areas, may have contributed to this trend (146).

Although ribotyping allows for the identification of specific strains, such as the hypervirulent ribotype 027, routine ribotyping is not performed in Costa Rican Social Security Hospitals. Ribotyping is typically reserved for research purposes or outbreak investigations. Our epidemiological analysis lacks strain specific data. Nevertheless, genotypic characterization is crucial for understanding the emergence of new strains and improving prevention strategies (147).

A 2009 outbreak in a major hospital in San José, Costa Rica identified NAP1 strain (45%) and the NAP_{CR1} strains (31%) as the predominate genotypes. Both strains showed resistance to ciprofloxacin, moxifloxacin and levofloxacin, NAP_{CR1} also exhibited resistance to clindamycin and rifampicin. A subsequent research about ribotyping that included a two -year period series without outbreaks (2010 to 2012), in a major Costa Rican hospital reported NAP_{CR1} genotype as the most prevalent and the coexistence of other 14 strains as NAP10 and NAP11 (148).

It is important to point out that the rate of CDI in a hospital is determinate not only by the risk of diseases among individual patient who carry the bacterium, but also by the risk of creating new carries through transmission from patients who are infected or colonized with *C.difficile* to those who are not (149).

In addition to the epidemiological characterization, our population-based study of HA-CDI included the description of groups at risk of infection as well as a description of potential risk factors, treatment received and clinical outcomes. In general, the prevalence of HA-CDI is more common in the group age of patients 55 years-old, which correlates with international evidence from Spain where CDI cases in patients of 50 years-old or older represented 87% of the total number of cases (142). The mean of age in our study was 55.7 years, which is also similar to another study from Turkey where the mean age was 56.4 years old. However, our mean is lower compared to studies from the United States and Europe. In a European cohort 63% CDI cases were developed in patients older than 65 years old (150).

Moreover, in our setting HA-CDI is more frequent in females (53%), according to the literature, some studies have reported higher CDI incidence in females compared with males for CA-CDI; however, is observed that for both CA- and HA-CDI, females appear to be at higher risk. The reason for the higher risk of CDI in females compared with males are still not clear (151). It has been proposed that sex-specific differences in the gut microbiome have been shown to be mediated by hormone levels, and transference of intestinal bacterial communities can alter sex hormone levels in animal studies (152).

In hospital care, *C.difficile* is commonly encountered in the ICU setting and critically ill patients are at significant risk for morbidity and mortality from this pathogen. Critically ill patients share many of the risk factors for developing severe CDI, so vigilance must be maintained in this patient population to prevent and rapidly treat the disease. Nevertheless only 3.9% of our CDI patients stayed in ICUs, there is a statistically significant association between nosocomial CDI and ICU stay, as demonstrated in the multivariable logistic regression for factors associated with CDI (ICU current admission, OR: 4.14 95% IC 1.79-0.97, p= 0.0009) (Table 7) (153,154).

Other departments in which CDI poses a problem are surgical wards, where perioperative antibiotic prophylaxis is applied and also treatment of healthcare-associated infections, including surgical site infections usually depend on the use of broad-spectrum antibiotics (153).

Another factor influencing CDI clinical outcomes like severity and in-hospital death is the length of stay (LOS). According to our PSM cohort CDI cases had longer mean of LOS than controls (33.2 days and 20.4days respectively), these findings compared to other studies demonstrate that our mean LOS is higher, as an example, in Spain HA-CDI LOS is around 26.2 days while in Mexico is around 20.1 days. Despite these differences, LOS in a CDI is considerably higher compared with average LOS in hospitals which is 8.3 days (145, 142).

LOS is particularly of interest in high complexity hospitals where more complicated patients are attended with enhanced length of hospitalization and more intensive and broader spectrum use of antimicrobials; characteristics frequently described as risk factors and determinants for CDI development and severity (155).

While many previous studies have evaluated the risk of antibiotics independently of other risk factors, this population-based study with PSM permitted the examination of the relative risk of antimicrobial and PPI consumption after adjustment for clinical risk factors, giving a more accurate picture of the role of antimicrobial consumption as the main dominant risk for HA-CDI.

The population-based observational approach of the case-control study may have led to selection bias. There were systemically significant differences in the following initial clinical parameters of patients between cases CDI and controls: hospital admission within 8 weeks, number of comorbidities, connective tissue diseases, chronic kidney disease, use of immunosuppressive agents and concomitant infectious disease. These differences in the baseline characteristics of patients are known in the literature to be associated with nosocomial CDI, which means that they can act as confounding factors in this proposed model of study that evaluates risk factors association with the development of the infection. Therefore, to minimize the risk of bias a PSM was conducted (1)

A PSM is a method of designing observational studies that mimic the characteristics of randomized controlled trials, allowing for a similar distribution of the observed baseline covariates between CDI cases and control groups (144).

For clinicians, early prediction of patients with CDI at risk of severe disease is important for decision-making and disease management. Identification of clinically significant risk factors is a major contribution to the infection approach. According to the literature, the most significant risk factor for CDI is antibiotic use. Antibiotic exposure alters the natural microbiota of the intestines allowing *C.difficile* to proliferate (46).

Antibiotics have long been established as the crucial risk factor for CDI. Previous studies have shown that use of antibiotics impacts individual risk for CDI when use of antibiotics is evaluated at the ward level, or even at the broader level of the hospital (156).

Although many antibiotics have been associated with CDI, the most commonly associated are clindamycin, fluoroquinolones, carbapenems and cephalosporins, these agents are known as high-risk antibiotics for CDI (157, 52). Antibiotic overuse has come under scrutiny as a major driver of CDI with 50% of inpatients prescribed an antibiotic during hospitalization and 30% of these antibiotics

being potentially unnecessary (158). Also, the use of multiple antimicrobials and prolonged antimicrobial therapy has been associated with an increased risk of CDI (52).

The association of CDI with a particular antimicrobial agent may depend on several factors, including the local prevalence of high resistance to antimicrobial agents in common use and the frequency with which the antimicrobial of interest is used (52).

Our antimicrobial consumption trend analysis (2017-2021) concluded that the most common antimicrobials consumed were cefotaxime, vancomycin, oxacillin and fluconazole, the high proportion of cefotaxime and third-generation cephalosporins in general may be explained by different reasons, for example, due to its broad-spectrum activity, cefotaxime is prescribed as an empirical treatment option. Trends analysis demonstrated that while a statistically significant increase in the consumption of carbapenems, trimethoprim-sulfamethoxazole, fluroquinolones vancomycin, echinocandins and azole antifungals were identified. In contrast, a statically significant decrease in consumption of cephalosporins and macrolides was reported (138).

As described before, a peculiar finding was the downtrend in cephalosporins consumption, a possible explanation is the substitution of cephalosporins with broad-spectrum antibiotics during the pandemic period, also, since 2018 ASPs have been in operation in the hospitals included in this study, promoting de-escalation and shortened therapies (138).

At ecological level, clindamycin, cephalosporins, macrolides and metronidazole are among the antimicrobials that showed a positive statistically significant association between consumption and nosocomial CDI incidence. A strong association was identified between nosocomial CDI with cephalosporins and clindamycin, even though these antibiotics show a downtrend in consumption.

Additionally, the population-based study found that following statistically significant associations between consumption and nosocomial CDI according to the adjusted risk ratio calculated in the matched cohort: number of antibiotics used (OR: 0.82 95%IC 0.73-0.92, p=0.0011), echinocandins (OR: 0.11, 95% IC 0.013-0.85, p=0.02), cephalothin (OR:0.51, 95%IC 0.28-0.91, p=0.03 and cefotaxime (OR: 1.61, 95% IC 1.15-2.25).

A case-cohort study of adult inpatient in Canada, measured the risk of HA-CDI associated with exposure to various antibiotic classes compared with patients with no antibiotic exposure in the 5 days prior to developing symptoms. After adjusting for age, gender, hospital exposure and infection pressure, the incidence of CDI was 2-3 times higher for penicillins, cephalosporins, fluoroquinolones and carbapenems (159).

According to the literature, one of the first outbreaks involving clindamycin-resistant *C.difficile* was reported in 1989. The strain predominant was highly resistant to clindamycin. Studies of these outbreaks established the increased risk of CDI with clindamycin consumption. At that time a decrease in the use of clindamycin in United States hospitals was identified, resulting in resolution of the outbreaks. Recently, it was seen that BI/NAP1 epidemic strain demonstrate variable susceptibility to clindamycin. This observation, combined with the fact that clindamycin is not used as commonly as other antimicrobials in adult patients, may explain why it has not been shown to be at the top of the list (52).

Furthermore, cephalosporins became a strong risk factor for CDI outbreaks probably because they became "workhorse agents" on hospital formularies. Use of second a third-generation cephalosporins such as cefotaxime and ceftriaxone is associated with a particularly high risk for CDI (159). Thus, tracking high-risk antibiotic use as a group is needed in time and volume-based assessments, to better inform stewardship in mitigating CDI risk (158).

Similar to cephalosporins, fluoroquinolones became popular antimicrobials for their good oral bioavailability and spectra of activity. The lack of association of fluroquinolones and CDI in our study may reflect changes in antibiotic use post ASPs implementation. From a statical perspective a lack of statistical significance for the association of a specific class of antibiotics and CDI does not necessary mean "no risk". Volume of use, the range of clinical characteristics in population, sample size, study design, among others may affect statistical test results (158). High-risk antibiotics monitoring could be a more practical stewardship intervention for CDI incidence and complications reduction where resources are limited.

Other risk factors included in this research model include the influence of PPIs. PPIs are one of the most prescribed groups of medicines globally, they are postulated to increase the proliferation of

spores and change the acid of the stomach that permits spores to survive intraluminally. The role of gastric acid suppression therapy has gained much interest recently as a risk factor for CDI, however, very low quality evidence in support of this association is found (56).

This study did not identify statistically significant changes in PPIs consumption trends and did not find correlations with nosocomial CDI incidence at ecological and population level, supporting the evidence that suggests a weak association between PPIs and CDI.

CDI clinical risk factors as comorbidities were assessed. The presence of various comorbidities is a confirmed risk factor for nosocomial CDI according to our case-control study OR: 0.88 95%IC 0.78-0.98, p=0.02. Comorbidities have long been considered potential risk factors for CDI, however, a systematic review and meta-analysis concluded that some comorbidities are of greater importance than others. Kidney diseases, liver diseases, cardiovascular diseases and bowel diseases are among the more associated with primary CDI (160).

Our study also describes the pharmacological approach and clinical outcomes of HA-CDI in population-based cohort. The primary difference in CDI treatment focuses on whether the infection is primary or recurrent episode, and the severity of the infection. This study included 96.6% new hospital onset cases, which required a first-line therapy. According to recent international guidelines updates, first-line treatment includes vancomycin or fidaxomicin, with metronidazole reserved for scenarios where those options are not available (66).

Even though there is strong evidence that supports fidaxomicin use for both initial and first rCDI episodes, with a lower recurrence rate than vancomycin and similar cure rates, it is an option not available in Costa Rica, for this reason it is not indicated in national guidelines, instead, oral metronidazole is still an option used in no severe HA-CDI episodes and CA-CDI. Oral Vancomycin is the preferred option according to our guidelines for non-severe episodes on the other hand for CDI severe or complicated cases oral vancomycin + IV metronidazole are used for 10 to 14 days. The use of alternative options as tigecycline usually is reserved as a rescue treatment for patients with ser CDI when treatment with vancomycin and metronidazole fails or is contraindicated. On the other, second recurrences are treated with vancomycin in pulsed or tapered doses (161).

Fecal Microbiota Transplantation is an option available in Costa Rica in refractory disease, studies have shown high cure rates, ranging from 80% to 95% in preventing CDI recurrence (162). FMT failure in this time series (2019-2021) is 30%, careful patient selection, donor screening and consideration of potential risks are essential for safe and effective treatment.

Finally, an all-cause and HA-ICD related mortality study was conducted in the matched cohort. In this cohort of patients with CDI, the most frequent cause of death or the one with the highest cumulative risk over time was due to causes other than CDI. This suggests that clinical management should adopt a comprehensive approach addressing not only infection control but also the associated comorbidities.

Strengths and limitations

Latin American courtiers do not regularly measure their antimicrobial consumption and only a few occasionally review the overall consumption of antibiotics withing their territory. Due to these inconsistences, data in this region are scare and usually spread. With this first multicentric study a standardized methodology that analyzed antimicrobial and PPIs consumption trends and their association with HA-CDI was implemented. Methodology for consumption was adopted from the WHO-DDD/ATC guidelines, which allows comparisons across time and healthcare facilities through the calculation of DDD/100 bed days and DDD/100 discharges. Also, this is the first study in the region that correlates CDI with hospital ward and population-based antibiotic consumption.

It is important to mention that this study is not without limitations. Firstly, we cannot be certain that the prescribing practices observed were representative of other hospitals of lower complexity in Costa Rica. Furthermore, other analyses of hospitals of different complexity, other reference hospitals and private institutions. Also, it was impossible to account for local antibiotic consumption CDI and specific strain epidemiology like NAP1 or NAP9, yet this could pose a significant impact on nosocomial CDI. In addition, our population-based study is non-randomized retrospective observational study, but through the propensity score matched analysis, we tried to reduce the potential bias and archive and analysis with similar cohorts. Finally, one of our main limitations was that environmental control of CDI infection was not part of our analysis, for this reason. Comprehensive antimicrobial stewardship efforts in conjunction with proper environmental

disinfection, hand hygiene compliances, appropriate isolation measures should be evaluated when analyzing characterizes of infection to stablish possible risk factors.

Recommendations for Future Research Lines

1. Future research should include primary, secondary, and private healthcare facilities to provide a more comprehensive national picture of *C. difficile* infection dynamics beyond tertiary care centers.
2. Integrating ribotyping or other molecular typing techniques into future studies would enable the identification of hypervirulent strains (such as NAP1, NAP9, and NAP_{CR1}), enhancing surveillance and outbreak response capabilities.
3. Assess the impact of specific antimicrobial stewardship interventions Prospective studies evaluating targeted antimicrobial stewardship strategies (de-escalation protocols, audit and feedback, local treatment guidelines) would help quantify their effectiveness in reducing HA-CDI incidence.
4. Research on environmental cleaning practices, hand hygiene compliance, patient isolation protocols, and healthcare worker contamination is essential to understand and mitigate nosocomial transmission pathways.
5. Further investigate the role of PPIs and other pharmacological agents. Although no strong association was found in this study, high-quality prospective studies or pharmacological analyses are needed to clarify the controversial link between PPIs and CDI risk.
6. Explore the effectiveness of emerging treatment options Studies on newer therapies such as fidaxomicin, bezlotoxumab, and FMT are needed to assess their clinical outcomes and feasibility in Latin American healthcare settings.

6

Conclusions

1 - This doctoral research constitutes one of the first multicenter studies in Latin America to comprehensively analyze the incidence, risk factors, and clinical features of healthcare-associated *Clostridioides difficile* infection (HA-CDI), as well as antimicrobial and proton pump inhibitor (PPI) consumption trends in tertiary care hospitals of the Costa Rican Social Security system (CCSS). Through a combination of ecological and population-based case-control approaches using propensity score matching (PSM), this study provides valuable insights for antimicrobial stewardship programs (ASPs) and infection prevention strategies in resource-limited settings.

2 - The study confirmed that the incidence of HA-CDI in the CCSS tertiary care hospitals between 2017 and 2021 was relatively low compared to other international contexts. However, significant clinical and epidemiological risk factors were identified, particularly advanced age, ICU admission, prolonged hospital stay, and the presence of multiple comorbidities. These factors underscore the need for early identification of high-risk patients and the implementation of tailored preventive interventions in hospital settings.

3 - Antibiotic exposure emerged as the most prominent and consistent risk factor for HA-CDI. Third-generation cephalosporins (notably cefotaxime), clindamycin, and other broad-spectrum agents showed statistically significant associations with increased CDI risk, even in the context of declining consumption trends. This highlights the importance of not only reducing unnecessary antibiotic use but also monitoring high-risk agents over time to guide stewardship decisions. Interestingly, some antibiotics such as echinocandins and cephalothin were associated with a lower risk, suggesting a nuanced relationship between antimicrobial class and CDI risk that warrants further exploration.

4 - In contrast to antibiotics, the role of PPIs in CDI development remains controversial. In this study, no statistically significant association was found between PPI consumption trends and HA-CDI incidence, supporting the emerging evidence of a weaker link than previously thought. Nonetheless, PPI use should still be evaluated critically in hospitalized patients due to its widespread prescription and potential interactions with other risk factors.

5 - The COVID-19 pandemic period provided a unique backdrop for part of this research. Despite increased antimicrobial consumption during the pandemic, the expected rise in CDI incidence was not observed. This paradox may reflect the complex interplay of altered hospital practices, enhanced infection control measures, and changes in patient demographics during the pandemic.

6 - This thesis also identified critical gaps in local epidemiology, particularly the absence of routine ribotyping in clinical laboratories. Incorporating molecular surveillance could enhance understanding of strain diversity and support outbreak prevention strategies. Furthermore, CDI-related outcomes—such as increased length of stay and all-cause mortality—emphasize the substantial clinical and economic burden of this infection.

7 - The use of PSM in the case-control study minimized bias and improved the internal validity of risk factor analysis, allowing for more accurate estimations of the attributable risk of antimicrobials and PPIs in the development of HA-CDI. However, limitations such as the lack of environmental data, molecular strain typing, and representation of hospitals with different complexity levels should be addressed in future research.

8 - Finally, this doctoral thesis provides robust evidence that antimicrobial use—particularly third-generation cephalosporins and clindamycin—is the main modifiable risk factor for HA-CDI in tertiary care hospitals in Costa Rica. Strengthening antimicrobial stewardship programs, optimizing antibiotic prescribing, and improving surveillance systems are essential steps to reduce CDI burden in the region. These findings contribute to the global understanding of CDI epidemiology in middle-income countries and offer a foundational framework for future research and public health interventions.

7

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8

Annexes

Annex 1: Poster presented at the Congress of the European Society of Clinical Microbiology and Infectious Diseases in April 2024

P0502

Changes in antibiotic consumption due to COVID-19 pandemic in tertiary-care hospitals in Costa Rica: A five year interrupted time series study

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A. Background

The COVID-19 pandemic poses changes in antimicrobial consumption (AMC) due to irrational antimicrobial use.
The aim of this study was to analyze the impact of COVID-19 pandemic on AMC in Tertiary Care Hospitals in Costa Rica according to the World Health Organization AWaRe classification.

B. Methods

Retrospective five year (2017 to 2021) interrupted time series study with AMC data from Tertiary Care Hospitals in Costa Rica (country of 5,1 million inhabitants) to access the impact of COVID-19 pandemic on AMC. Simple and segmented linear regression models were used to analyze trends and level changes in both overall hospital and ICU. The time series are divided into 2 segments, the first point is March 2020 when COVID-19 pandemic started and July 2020 which is the month where COVID-19 incidence began to increase according to the Ministry of Health statistics of Costa Rica. Antimicrobial consumption was measured in monthly defined daily doses (DDD) per 100 bed days (DDD/100 BD). P values of <0.05 were considered statistically significant.
The ethics committee CEC-CENTRAL-CCSS reviewed and approved the study on May 2023 (registry number R022-SABI-00319).

C. Results

The overall hospital AMC according to AWaRE classification did not report statistically significant changes in the two segments. In ICU analysis statistically significant changes were seen at the first segment of the time series.

The Access group showed an increase of 313.81 (95% CI 176.98 to 450.64, p<0001), followed by a negative trend of -21.07 per month (95% CI -30.31 to -11.84, p<0001). The Watch group had an increase of 435.57 (95% CI 172.09 to 699.05, p=0.0016) and a trend of -43.08 (-60.86 to -25.29).

In the Reserve group the change level was 53.06 (95% IC 23.53 to 82.60, p=0.0007) and a trend change of -4.19 (95% CI -6.18 to -2.20, p<0001) (Table 1, Fig 1 and Fig 2).

Table 1 Results of the interrupted time series analysis of changes in Overall Hospitals and Intensive Care Units antibiotic consumption in Tertiary Care Hospitals in Costa Rica between 2017 and 2021 according to the WHO Aware Classification

Antibiotic agent (application)	Baseline antibiotic consumption in DDD/100BD (95% CI) (B0)	Baseline trend in DDD/100 BD per month (95%CI)	Level change at the begging of COVID-19 pandemic in DDD/100 BD (95%CI)	Trend change at the begging of COVID-19 pandemic in DDD/100 BD per month (95% CI)	Level change at first COVID-19 incidence increase in DDD/100 BD (95% CI)	Trend change after the first COVID-19 incidence increase DDD/100 BD per month (95% CI)
WHO AWaRe Classification Overall Hospital						
Access	4013.26 (3569.20 to 4457.32) p<0001	-6.97 (-26.82 to 10.40) p=0.43	18.20 (-711.83 to 748.22) p=0.96	10.82 (-38.45 to 60.08) p=0.66	-55.18 (-828.58 to 718.22) p=0.89	20.64 (-42.53 to 83.80) p=0.51
Watch	3686.22 (3247.88 to 4124.55) p<0001	3.60 (-14.16 to 21.36) p=0.68	306.10 (-454.53 to 1066.74) p=0.42	-16.53 (-67.86 to 34.80) p=0.52	-29.05 (-94.88 to 36.76) p=0.37	-29.06 (-94.88 to 36.76) p=0.38
Reserve	216.53 (166.85 to 266.20) p<0001	2.22 (0.20 to 4.23) p=0.03	31.52 (-56.06 to 119.11) p=0.47	0.12 (-5.79 to 6.03) p=0.97	-71.51 (-162.85 to 19.82) p=0.12	5.14 (-2.32 to 12.60) p=0.17
WHO AWaRe Classification Intensive Care Units						
Access	242.97 (155.13 to 330.81) p<0001	3.14 (-0.41 to 6.70) p=0.08	313.81 (176.98 to 450.64) p<0001	-21.07 (-30.31 to -11.84) p<0001	127.10 (0.38 to -34.40) p=0.12	-23.17 (-36.36 to -9.98) p=0.0009
Watch	486.45 (330.04 to 642.85) p<0001	211.39 (-76.18 to 498.96) p=0.15	435.57 (172.09 to 699.05) p=0.0016	-43.08 (-60.86 to -25.29) p<0001	211.39 (-76.18 to 498.96) p=0.15	-50.93 (-74.42 to -27.44) p<0001
Reserve	31.04 (12.70 to 49.38) p=0.0013	0.97 (0.23 to 1.72) p=0.01	53.06 (23.53 to 82.60) p=0.0007	-4.19 (-6.18 to -2.20) p<0001	8.14 (-25.58 to 41.87) p=0.63	-4.00 (-6.76 to -1.25) p=0.005

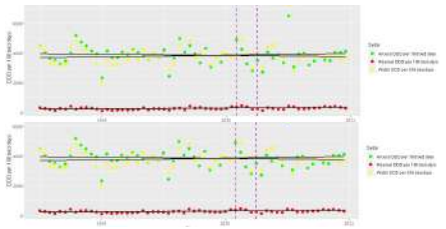

Fig 1. Consumption of AWaRe antibiotics in Overall Hospital in Tertiary Care Hospitals In Costa Rica

Fig 2. Consumption of AWaRe antibiotics in ICU in Tertiary Care Hospitals In Costa Rica

D. Conclusion

There was a statistically significant increase in the antibiotic consumption in the ICU when COVID-19 pandemic started, specially noticed within the Watch group.
The fact that this group was the most affected is worrisome particularly because it includes many of the agents that have a greater impact on AMR and that should be prioritized in AMS programs. The causes that triggered an increase in the use of antimicrobials during the period after the global declaration of the pandemic should be analyzed in depth.

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P3383

Relationship between high-risk antibiotic consumption and incidence of nosocomial *Clostridiodes difficile* infection in Tertiary Care Hospitals in Costa Rica during COVID-19 pandemic

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A. Background

It is known that antimicrobial use is one of the most important modifiable risk factors for the development of *Clostridiodes difficile* infection (CDI). It has been widely described that during the COVID-19 pandemic there was an increase in the use of antibiotics.

Antibiotics such as penicillins, cephalosporins, carbapenems, fluoroquinolones and clindamycin are considered high risk for the development of CDI.

The aim of the study was to analyze whether the evolution in nosocomial CDI rates in Tertiary Care Hospitals in Costa Rica could have any relationship with previously defined high-risk antimicrobial consumption (HRAC) during COVID-19 pandemic.

B. Methods

Retrospective interrupted time-series analysis from January 1st 2017 and December 31st 2021 of antimicrobial consumption and nosocomial CDI monthly rate were performed using data of monthly HRAC in Defined Daily Doses (DDD)/100 beds from participating hospitals databases. Segmented Poisson regressions were used to assess whether variations in rates of nosocomial CDI followed variations in HRAC. The breakpoint in these time-series analysis is July 2020 which is the month where COVID-19 incidence began to increase according to the Ministry of Health statistics of Costa Rica. P values of <0.05 were considered statistically significant.
The ethics committee CEC-CENTRAL-CCSS reviewed and approved the study on May 2023 (registry number R022-SABI-00319).

C. Results

A total of 701 nosocomial CDI cases were reported and 55679 DDD per 100 bed-days of high-risk antimicrobials were distributed in the participating hospitals during the study period (Fig 1 and Fig 2). Table 1 summarizes segmented Poisson regression results. Changes in HRAC did not show statistically significant variations in the rate of CDI at breakpoint. Major changes were seen with Carbapenems consumption 0.0013 (95% CI -0.023 to 0.016) level change -0.95 (95% CI –12.33 to 4.67) and trend change 0.057 (95% IC -0.59 to 0.80) and Penicillins consumption 0.0004 (95% CI -0.016 to 0.012), level change -0.91 (95% IC -12.33 to 4.95) and trend change 0.045 (95% IC -0.64 to 0.78).

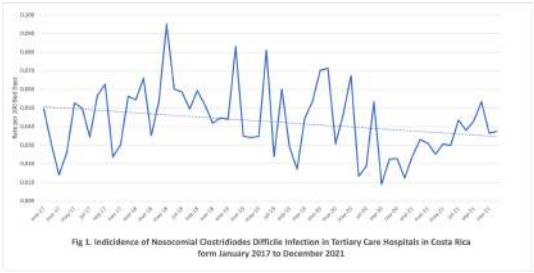

Fig 1. Indicidence of Nosocomial Clostridiodes Difficile Infection in Tertiary Care Hospitals in Costa Rica form January 2017 to December 2021

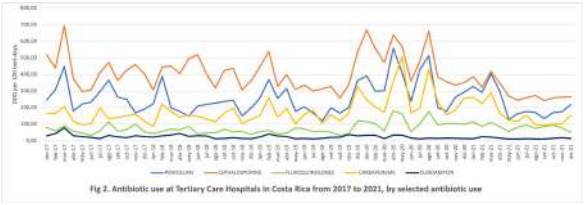

Fig 2. Antibiotic use at Tertiary Care Hospitals in Costa Rica from 2017 to 2021, by selected antibiotic use

Table 1 Results of the interrupted time series analysis at identified breakpoint

Antimicrobial group	Antimicrobial use at Breakpoint in DDD/100 BD (95% CI)	Level change at first COVID-19 incidence increase in DDD/100 BD (95% CI)	Trend change after the first COVID-19 incidence increase DDD/100 BD per month (95% CI)
Cephalosporins	0.0011 (-0.0136 to 0.0124) p=0.86	-0.91 (-12.30 to 4.60) p=0.98	0.059 (-0.59 to 0.78) p=0.84
Carbapenems	0.0013 (-0.023 to 0.016) p=0.89	-0.95 (-12.33 to 4.67) p=0.78	0.057 (-0.59 to 0.80) p=0.85
Clindamycin	0.0006 (-0.15 to 0.096) p=0.99	-0.87 (-12.34 to 4.60) p=0.79	0.039 (-0.58 to 0.77) p=0.88
Fluroquinolones	0.0037 (-0.050 to 0.041) p=0.86	-1.01 (-12.53 to 4.92) p=0.77	0.054 (-0.57 to 0.80) p=0.84
Penicillins	0.0004 (-0.016 to 0.012) p=0.96	-0.91 (-12.33 to 4.95) p=0.79	0.045 (-0.64 to 0.78) p=0.87

D. Conclusion

Non-significant increases were observed in antibiotics considered high risk for CDI acquisition. This fact questions its possible relationship with the decrease in nosocomial CDI in Tertiary Care Hospitals in Costa Rica during the first months of the COVID-19 pandemic. Further studies are needed to understand other factors implicated in the change in epidemiology of nosocomial CDI.

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Annex 3: Poster presented at the Congress of the Spanish Society of Infectious Diseases and Clinical Microbiology in May 2024

30 MAYO AL 1 DE JUNIO 2024 ZARAGOZA

Tendencias del consumo de antimicrobianos en hospitales del tercer nivel de atención en Costa Rica de 2017 al 2021

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Introducción:

La cuantificación del consumo de antimicrobianos es una de las actividades descritas en los Programas de Optimización de Antimicrobianos (PROA). Su función es detectar puntos susceptibles de acciones de mejora y evaluar el impacto de intervenciones a través de la comparación entre hospitales del mismo nivel y el análisis de series temporales. La Dosis Diaria Definida (DDD) es la unidad de medida oficial establecida por la Organización Mundial de la Salud (OMS) (Clasificación Anatómica Terapéutica (ATC)/DDD) para cuantificar consumo de antimicrobianos permitiendo obtener datos estandarizados de consumo que pueden compararse entre diferentes centros hospitalarios a través del tiempo.

Objetivo:

Analizar si las tendencias de consumo de antimicrobianos en hospitales del tercer nivel de atención de Costa Rica durante el periodo 2017 al 2021 expresadas en DDD/100 estancias siguen el mismo patrón que cuando se expresan en DDD/100 altas.

Metodología:

Estudio retrospectivo observacional descriptivo de cuantificación del consumo antimicrobiano utilizando los datos de dispensación de los Servicios de Farmacia disponibles, dos de los tres hospitales generales de tercer nivel fueron incluidos en el estudio. Se aplicó la metodología ATC/DDD de la OMS para realizar el cálculo de cuantificación. Los antimicrobianos fueron clasificados en antibióticos (J01), antifúngicos (J02) y antivirales (J05). Se obtuvo los promedios anuales de consumo mediante el cálculo de las DDD/100 estancias y DDD/100 altas. La última versión (2023) de la clasificación de DDD se utilizó para los 5 años. Las tendencias del consumo de antimicrobianos fueron analizadas utilizando un modelo de regresión logística lineal generalizado con cada grupo de antimicrobianos, valores de $p< 0,05$ se consideraron estadísticamente significativos. El análisis estadístico se realizó utilizando el programa SAS® Studio OnDemand for Academics (SAS® Institute Inc, Estados Unidos).

Resultados:

Se observó un aumento estadísticamente significativo del consumo promedio de antifúngicos (J02), en DDD/100 estancias: 26,30 (2017) a 32,49 (2021) (p=0,05) y DDD/100 altas: 80,41 (2017) a 123,52 (2021) (p<0.0001) (Ver Tabla 1).
El análisis de los grupos específicos de antimicrobianos dio lugar a la observación de una disminución en la tendencia de consumo de cefalosporinas no estadísticamente significativa de DDD/100 estancias: 430,66 (2017) a 296,08 (2021) (p=0,29) y estadísticamente significativa de DDD/100 altas 2595,15 (2017) a 1946,72 (2021) (p<0.0001).
Por otra parte, se observó un aumento no estadísticamente significativo de DDD/100 estancias de carbapenémicos: 146,13 (2017) a 156,77 (2021) (p=0,27) y estadísticamente significativo de DDD/100 altas: 699,00 (2017) a 948,00 (2021) (p<0,0001). Con trimetoprim-sulfametoxazol igualmente se observó una tendencia al aumento de DDD/100 estancias no significativo: 74,42 (2017) a 107,19 (2021) (p=0.06), mientras que con DDD/100 altas la tendencia fue estadísticamente significativa: 475,60 (2017) a 772,85 (2021) (p<0,0001). Con respecto a las equinocandinas se observó una tendencia a la disminución no significativa de DDD/100 estancias: 116,52 (2017) a 163,64 (2021) (p=0,59), mientras que con DDD/100 altas la tendencia al aumento fue estadísticamente significativa (p=0,01): 156,94 (2017) a 189,48 (2021). Solamente los macrólidos y antifúngicos azólicos evidenciaron una disminución estadísticamente significativa tanto en DDD/100 estancias como en DDD/100 altas, obteniéndose los siguientes resultados con macrólidos: DDD/100 estancias: 50,42(2017) a 25,39(2021) (p=0,005) y DDD/100 altas: 360,60 (2017) a 174,26(2021) (p<0,0001) y antifúngicos azólicos: DDD/100 estancias: 116,52(2017) a 163,64(2021) (p=0,0008) y DDD/100 altas: 647,18 (2017) a 1045,72 (2021) (p<0,0001).

Tabla 1 Tendencias globales del consumo de antimicrobianos (J01, J02, J05) en Hospitales del Tercer Nivel en Costa Rica desde 2017 al 2021 expresado en DDD/100 camas día y DDD/100 altas

Total	2017	2018	2019	2020	2021	%	Tendencia	Valor P
Total de antimicrobianos (J01, J02, J05)								
DDD/100 estancias	61,35	54,30	60,13	93,05	61,78	0,70	3,96	0,31
DDD/100 altas	270,63	245,68	277,80	295,44	313,25	15,75	13,50	0,0007
Total de antibióticos (J01)								
DDD/100 estancias	30,36	27,92	28,67	40,86	25,51	-15,97	0,32	0,84
DDD/100 altas	164,29	149,18	155,53	154,64	161,92	-1,44	0,072	0,96
Total de antifúngicos (J02)								
DDD/100 estancias	26,30	22,68	27,60	43,21	32,49	23,53	3,29	0,05
DDD/100 altas	80,41	73,59	94,49	104,61	123,52	53,61	11,72	<0,0001
Total de antivirales (J05)								
DDD/100 estancias	4,69	3,70	3,87	8,98	3,77	-19,61	0,34	0,57
DDD/100 altas	25,93	22,91	27,78	36,19	27,81	7,25	1,70	0,14

Conclusión:

El conocimiento de las tendencias del consumo de antimicrobianos tanto a nivel local como regional es esencial para conocer los patrones de uso de los antimicrobianos utilizados de forma usual, así como los de nueva inclusión en el Listado Terapéutico de los hospitales.
En nuestro caso de estudio se determinó que las tendencias de consumo de antimicrobianos expresados en DDD/estancias como en DDD/altas siguen un patrón similar, excepto para los antifúngicos azólicos. El conocimiento de las tendencias de consumo de antimicrobianos especialmente antes y después de la pandemia podrían ser de utilidad para los PROA al determinar posibles patrones de prescripción y su eventual repercusión en la resistencia a los antimicrobianos.

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