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Review Article

Clostridium difficile Infection (CDI) by Hypervirulent BI/NAP1/027 Strain: a Comprehensive Review of Toxigenicity, Pathogenesis, Risk Factors, and Preventative Measures

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ABSTRACT

Clostridium difficile is an anaerobic bacil gram-positive bacteria, able to form spores and toxin, that is transmitted among humans through the fecal–oral route. Clostridium difficile infection (CDI), a typical nosocomial infection has been contributed to a significant proportion of morbidity and mortality among in-patients with a case-fatality rate of 14% within 30 days after diagnosis. Profound culture and toxin examination for C. difficile are still minimal in many hospitals in various Asian countries. Consequently, C. difficile reports in Asia remain rare. Highly virulent form of C. difficile caused greater fatality and epidemics severity. Elderly age, hospitalization, exposure to antibiotics e.g., cephalosporins, fluoroquinolones, clindamycin, and penicillin contributed as main risk factors. Hypervirulent strain BI/NAP1/027 demonstrated to carry CdtLoc gene locus encodes CD196 ADP-ribosyltransferase (CDT) or known as binary toxin. Virulence factors are TcdA, TcdB, CDTa CDTb in which hypersporulation and mutation of TcD gene by hypervirulent strain led to toxin hyperexpression. Early cases detection, building management team to evaluate patient positive with all C. difficile toxins, hand hygiene improvement, continuation of contact precautions after diarrhea resolution, audit of infection control, and restriction of antimicrobials should be implemented as preventative measures. Focus measures also should emphasize on development of vaccine of C. difficile to boost immune state of elderly people. This review aims to describe severity of disease caused by hypervirulent BI/NAP1/027 C. difficile strain, its mechanism or pathogenesis, risk factors, current treatment options available, along with proposed preventative measures and infection control.

***Keywords**: Clostridium difficile infection (CDI), hypervirulent strain, BI/NAP1/027*

ABSTRAK

Clostridium difficile adalah bakteri basil gram positif anaerobik, pembentuk spora dan toksin, yang ditularkan di antara manusia melalui rute fekal-oral. Clostridium difficile infection (CDI), sebuah tipikal infeksi nosokomial telah berkontribusi pada proporsi yang signifikan terhadap morbiditas dan mortalitas di antara pasien rawat inap dengan tingkat fatalitas kasus 14% dalam waktu 30 hari setelah diagnosis. Kultur dan pemeriksaan toksin C. difficile masih minim di banyak rumah sakit di berbagai negara Asia. Akibatnya, laporan C. difficile di Asia masih jarang. Epidemi kematian dan keparahan yang lebih besar dari CDI disebabkan oleh C. difficile yang hipervirulen. Faktor risiko utama adalah usia lanjut, rawat inap, paparan antibiotik misalnya sefalosporin, fluoroquinolones, klindamisin, dan penisilin. Strain hipervirulen BI/NAP1/027 terbukti membawa lokus gen CdtLoc yang mengkode CD196 ADP-ribosyltransferase (CDT) atau dikenal sebagai toksin biner. Faktor virulensi yaitu TcdA, TcdB, CDTa CDTb; strain hipervirulen mampu melakukan hipersporulasi dan mutasi gen TcD yang menyebabkan hiperekspresi toksin. Tindakan pencegahan dapat dilakukan dengan deteksi dini kasus, pembentukan tim manajemen untuk mengevaluasi pasien yang positif semua toksin C. difficile, peningkatan kebersihan tangan, kelanjutan tindakan pencegahan kontak setelah resolusi diare, audit pengendalian infeksi, dan pembatasan antimikroba. Fokus upaya juga sebaiknya ditekankan pada pengembangan vaksin C. difficile untuk meningkatkan

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status kekebalan pada individu berusia lanjut. Tinjauan ini bertujuan untuk menggambarkan tingkat keparahan penyakit yang disebabkan oleh strain C. difficile BI/NAP1/027 hipervirulen, mekanisme atau patogenesisnya, faktor risiko, pilihan pengobatan yang tersedia, serta tindakan pencegahan dan pengendalian infeksi.

Kata kunci: *Clostridium difficile infection (CDI), strain hipervirulen, BI/NAP1/027*

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INTRODUCTION

Clostridium difficile infection (CDI) has been known as a typical nosocomial infection and contributes to a significant proportion of morbidity and mortality among in-patients with a case-fatality rate of 14% within 30 days after diagnosis.[1] *C. difficile* gives rise to numerous infections varying from mild diarrhea to pseudomembranous colitis (PMC), mainly in elderly patients with antibiotic treatment. In addition, high healthcare costs related to CDI increase the financial burden of government on health expenditure. It was recorded that half a million infections were attributed to CDI in the United States in 2011 with an incidence rate of 8.75 cases/1,000 adult admissions in 2009.[2,3] A literature study by Collins et al.[4] found few data of CDI cases. The study found that a study in Japan only reported on the ribotyping result of *C. difficile* without any information on CDI prevalence or incidence in Japan; CDI incidence increased from 1.7/1,000 to 2.7/1,000 adults in Korea, and 17.1/10,000 inpatients in Shanghai were attributed to CDI. Meanwhile, about 44% and 14% of colitis positive patients were positively diagnosed with the *C. difficile* toxin in Philippine and Malaysia, respectively.[4] A more recent study showed that CDI prevalence was 9.2% in Thailand.[5] There are only a few reports about CDI incidence or prevalence in Indonesia. A study reported that there were eight types of *C. difficile* strains presenting in healthy people,[6] while another study showed that the prevalence of *C. difficile* (toxin A) was 1.3% in community and hospital in Jakarta.[7] The last report originated from Central Java showing the prevalence of CDI to be 20.6% by 2017.[8]

Profoundly extensive culture and toxin examination for *C.difficile* are still minimal in many hospitals in various Asian countries. Consequently, *C.difficile* reports in Asia remain rare. In the current study held in Malaysia, assays determining toxin A/B from 175 stool samples collected from patients with antibiotic-associated diarrhea have been performed in tertiary hospital in north-eastern suburb; 24 of them (13.7%) tested positive for toxin, where the age most of infected patients is >50 years.[9-11] However, no ribotyping or any other molecular test have been done in regard to isolates of Malaysian *C. difficile*. Similar to Malaysia, CDI cases reporting in Indonesia is uncommon. It has been found 1.3% test results of stool sample reveal the etiology of diarrhea in Indonesia children was *C. difficile*. Furthermore, only enzyme immunoassay of toxin A was conducted; therefore, the *C. difficile* true prevalence may have been substantially greater. Molecular study of eight isolates collected from Indonesia established five of the results identified as toxinotype VIII and ribotype 017, assembled into epidemic strains of international 017. Two of them are A+B+ toxinotype 0, and one remaining A-B+ isolate was identified as toxinotype XVI binary toxin.[12-14]

Some risk factors including advanced age, antibiotic exposure, and hospitalization are highly associated with CDI.[15] Regulation of antibiotic usage in Asian countries is considered to be poor. There has been a review in Southeast Asian countries which depicted 47% of pneumonia cases as not receiving proper antibiotic whereas 54% of patients with diarrhea were receiving antibiotic unnecessarily, with 40% of under-dose antibiotics prescribed. The advanced age individuals with recent antibiotic treatment are at

the highest risk for CDI as they lack of beneficial gut microbiota and have low immunity due to age and other comorbidities.[16-20] This group is excessively affected and has the highest mortality rate due to CDI with 2% of risk increase every year after 18 years old of age. A report described around one in ten deaths due to CDI in advanced age people in the USA in 2010. There were no data of CDI on advanced age people in Indonesia, which could be due to lack of surveillance on CDI cases followed by limited laboratory facility in the hospital capable of diagnosing CDI. Besides, recurrence (relapse/reinfection) and death cases due to CDI in advanced age people would be higher because of improper treatment.[21]

Severe form of *C. difficile* infection (CDI) is caused by hypervirulent strain identified as type 1 of North American pulsed field, type B1 class from analysis of restriction-endonuclease, ribotype 027 as presented by PCR. Hypervirulent strain leads nationwide CDI outbreaks in European countries, Canada, and the United States (U.S.). First outbreak report of type 027 CDI was in Canada where the worst infected was Quebec in 2005. In the U.S., type 027 CDI affected 38 states. Meanwhile based on European Centre for Disease Prevention and Control, there were infections in 16 countries due to type 027 CDI. Hypervirulent strain of toxinotype III nurture TcdA and TcdB toxin genes, possess deletion of 18-bp in TcdC of toxin regulatory gene, and deletion at area 117. It leads to premature stop codon and frameshift, causing TcdC protein truncation. The rising case of type 027 virulence associated with more excessive toxin production can be attributed to lack control of regulatory TcdC.[19-21]A cohort study estimated that around 40% of CDI cases were community-acquired CDI (CA-CDI). CA-CDI occurs in younger age people, less severe symptoms, shorter hospital stay, lower recurrence rate and no deaths have been reported attributable to CA-CDI. Besides, CDI is also exacerbated by the discovery of hypervirulent strains and antibiotics resistant to quinolones, gatifloxacin instead of levofloxacin.[17-19] The emerging of CA-CDI will be a risk factor for domestic and foreign tourists who visit Bali.

Since 2000, greater fatality and severity epidemics of CDI have been caused by a highly virulent form of *C. difficile*. BI/NAP1/027 strains have spread widely and robustly over past 10 years and have been associated to CDI epidemics. The prevalent ribotypes in the Middle East are 140, 126, 078, 046, 014, 002, and 001, meanwhile the more prevalent ribotypes in Asia are 018, 017, 014, 002, and 001. In North America and Europe, ribotypes 078, 027, 020, 014, and 001 have been the uppermost strains.[22-24] Ribotype 027 has been found to possess reduced susceptibility to chloramphenicol, imipenem, clindamycin, moxifloxacin, rifampicin, and metronidazole. These characteristics implicate to more severe presentation of disease, high morbidity and mortality rates due to antimicrobial resistance juxtaposed to other strains. Spores of ribotype 027 expand more robustly and easily in hospital as they able to resist disinfectants, cleaning, and hospital surroundings. Observational study on patients with diarrhea in Veteran Affairs Medical Center, U.S. demonstrated around 22% of them were positive of BI/NAP1/027 strain.[19-24] This literature review aims to describe severity of disease caused by hypervirulent BI/NAP1/027 *C. difficile* strain, its mechanism or pathogenesis, risk factors, current treatment options available, along with proposed preventative measures and infection control.[22-24]

Clostridium difficile INFECTION (CDI)

Clostridium difficile is an anaerobic gram-positive bacillus bacterium, able to form spore and toxin, transmitted in humans by fecal–oral pathway. In the U.S., *C. difficile* is the most frequent nosocomial pathogen reported. A surveillance study of 2011 found 453,000 CDI cases with 29,000 associated deaths, wherein around a quarter of those were community-acquired. Nosocomial *C. difficile* infection quadruples hospitalizations cost causing rise of expenditures by about $1.5 billion in the U.S. yearly. It was recorded that half a million infections were attributed to CDI in the United States in 2011 with an incidence rate of 8.75 cases/1,000 adult admissions in 2009. In Hong

Kong, there were more than fifteen thousand CDI cases from 2006 to 2014 in which most were identified as a nosocomial infection. A nationwide study in Korea revealed CDI total incidence was 2.7 cases/1,000 adult admissions in 2008. CDI is also known for its propensity to recurrence among 35% of patients with antibiotic therapy and more than a half of recurrences of CDI are identified as relapse (relapse or reinfection).[25]

Due to CDI, approximately $1.1 billion are utilized in healthcare cost annually in the USA, while about €3 million is associated with healthcare costs in Europe. Compared to reports from countries across Europe and the USA, the prevalence of CDI in Asia is not fully known. In Korea, survey across 17 tertiary hospitals, from 2004 until 2008, found CDI incidence cases soared from 1.7/1,000 to 2.7/1,000 adults. Community-acquired CDI (CA-CDI) proportion over total CDI cases in a hospital in Busan was 7.1%, meanwhile 59.4% of CDI cases at a Seoul hospital's emergency department were CA-CDI. Based on a comprehensive study in Shanghai, China from March 2007 until April 2008, overall CDI incidence was 17.1/10,000 admissions; mild CDI because of younger mean age (62.8 years) compared to 63% patients were ≥65 years in a comprehensive European study. In addition, a survey across 13 Asia-Pacific countries demonstrated the proportion of CDI associated to healthcare facility was 53.6% and CA-CDI was 16.5%.

CDI case reports in Indonesia remain uncommon. *C. difficile* was identified in 1.3% stool samples of Indonesian children. However these data were not enough to reflect global prevalence in Asia. Furthermore CDI prevalence data of elderly are still unavailable to date.[22-26]

CDI mostly occurs in advanced age people, which is possibly explained by some of the risk factors, including frequent exposure to healthcare, age-related changes in physiology, increasing antibiotics usage, changes in the composition of gut flora, and increased comorbidities. Frequent exposure to healthcare increases the opportunity of contacting with environments contaminated with endospores of *C. difficile* and frequent utilization of antimicrobials. Carriers of *C. difficile*, both asymptomatic and symptomatic, could contain spores on their skin and discard those into the environment. Age-related physiologic changes also increase the risk of CDI, particularly changes in the immune system. The development and recurrence of CDI have been associated with the ability to generate immune responses, and the ability to produce antibodies against toxin may affect the progress of colonization and active infection. Aging is accompanied by immune senescence – a degeneration of the immune system related to advanced age – and it has been associated with a diminishing adaptive immune system.[27-29]

C. difficile has the ability to do colonization in large intestine, then releasing exotoxins protein (TcdA, TcdB) leading to colitis in people with risk factors. Figure 1 depicts TcdA and TcdB arbitrate *C. difficile* diarrhea, causing Rho family members' inactivation, Rho GTPases (guanosine triphospatases). This is followed by neutrophilic colitis, colonocyte death, functional loss of intestinal barrier, and death of colonocytes. Expression of clinical CDI disease is exerted by host immune responses and strain of *C. difficile*. A dramatic increase of severe CDI in hospitals was initially reported in the beginning of 2000s. CDC (Centers for Disease Control and Prevention) depicted isolates were group BI of restriction endonuclease, NAP1 (gel electrophoresis of North American), and PCR (polymerase chain reaction) 027; therefore, as BI/NAP1/027. This strain's characteristics are high level resistance of fluoroquinolones, robust production of toxin, efficient rate of sporulation, and significantly high mortality compared to less virulent *C. difficile.*[28,29] BI/NAP1/027 strain firstly originated in North America and Western Europe, but currently it spreads to various settings of hospitals across the globe.[30,31]

Even though hospital-acquired CDI has been the majority, CA-CDI has been increasing significantly and contributes to a third of new CDI cases. CA-CDI happens when onset of disease begins within 12 weeks in individuals who did not stay overnight in hospitals or other healthcare facility. CA-CDI could occur in younger patients, who have unclear antibiotics

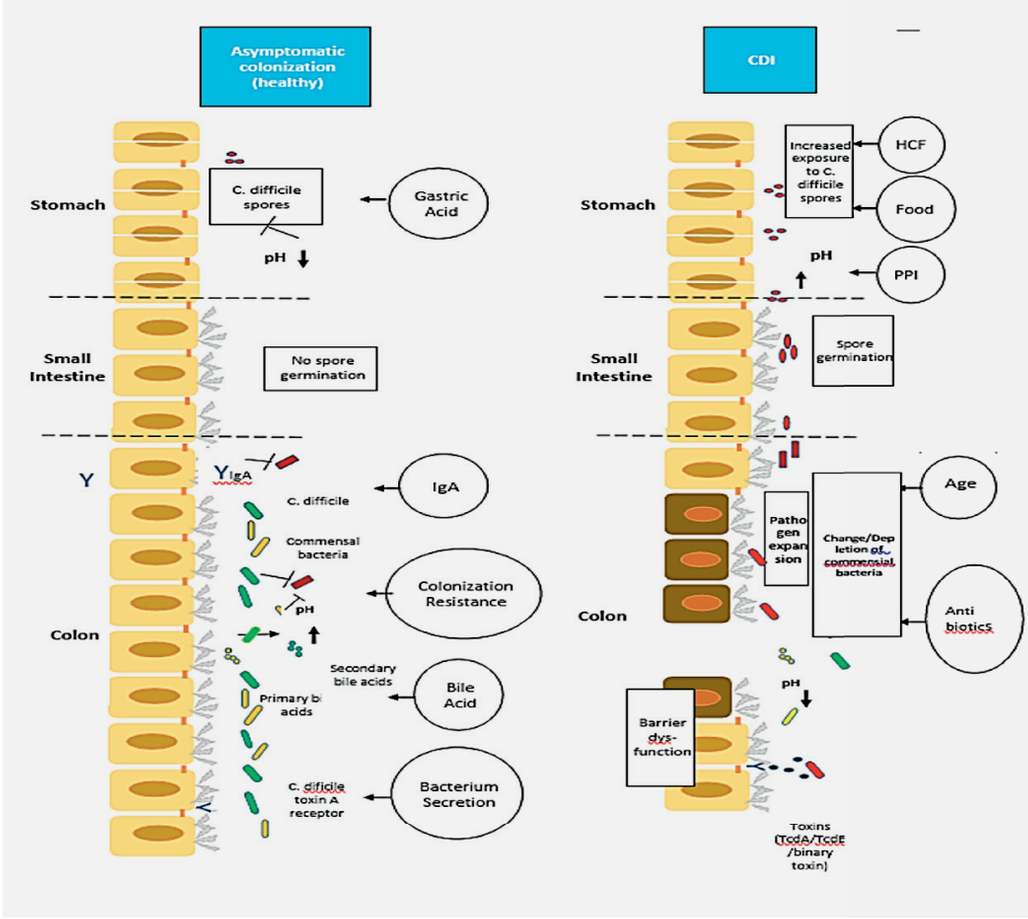

Figure 1. Progress from asymptomatic colonization to *C. difficile* infection (CDI)[22-29]

exposure and unknown risk factors. Therefore, CA-CDI acquisition main modes are currently in investigation. CA-CDI associated morbidity and mortality remain lower compared to hospital-acquired CDI. Nonetheless, 40% of CA-CDI patients need hospitalization and relapse rates are similar to HA-CDI.[32] Acid suppression influence to CDI remains unclear. Theoretically, gastric acid suppression allows more vegetative organisms to reach colon. However, *C. difficile* produces spores resistant to acid pH.[33,34]

HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN RISK FACTORS

Substantial risk factors of CDI by BI/NAP1/027 strain are namely hospitalization, elderly age, and exposure to antibiotics, e.g., cephalosporins and fluoroquinolones. Particular fluoroquinolones identified being risk factors are ciprofloxacin, gatifloxacin, moxifloxacin, and levofloxacin, presumed as consequences of fluoroquinolones-resistance in endemic strain. Almost all antibiotics of cephalosporin class are resistant to all *C. difficile* types and have been incriminated as significant risk factor in hospitals where endemic strain exists, as well as its usage for surgical prophylaxis. Consumption of agents to lower stomach acid production, e.g., proton pump inhibitors (PPI), and type 2 blockers of histamine have been recognized inconsistently as CDI risk factors in hospital with predominance of endemic strain. Besides resistance to current fluoroquinolones, other specific factors of BI/NAP1/027 strain dissemination as well as severe CDI caused by this hypervirulent strain remain speculative and need to be the substance of thorough study or research.[32-34]

Administration to almost all groups of antimicrobials has been delineated to cause CDI, even though cephalosporins, clindamycin,

penicillin, and present fluoroquinolones are notably reported as common culprits. Numerous isolates of *C. difficile* were found containing elements of mobile genetic, markers of antibiotic resistance in chromosomes, and mutations of genetic conferring resistance into rifamycins, chloramphenicol, tetracyclines, streptogramin, lincosamide, macrolide, and fluoroquinolones. Patients also progress to CDI disease following antibiotics therapy which leads to susceptibility of *C. difficile* strain infection. Presumably, CDI occurs due to suppression of normal microbiota in intestine for extended periods after discontinuation of antibiotics; therefore, allowing sustained opportunity for colonization and infection of *C. difficile*. Isolates resistance to clindamycin, erythromycin frequently associated to epidemics and outbreaks. Furthermore, individuals administered by clindamycin possess a remarkable high-rise CDI frequency caused by clindamycin resistant. Resistance is commonly associated with erm(B) presence encoding methyltransferase and ground in Tn5398 conjugative transposon.[35] Current exploration of BI/NAP1/027 isolates has exemplified re-emergence of erythromycin-resistant BI/NAP1/027 in European countries, the U.S., and Canada.

Currently, therapy with fluoroquinolones is identified as BI/NAP1/027 *C. difficile* infection risk factors, and there is a proposed association between therapy with fluoroquinolones and emergence of BI/NAP1/027 strain. Even though all previous isolates of BI/NAP1/027 are susceptible to gatifloxacin, moxifloxacin, and fluoroquinolones, yet resistant to levofloxacin and ciprofloxacin, almost all current isolates were resistant to all fluoroquinolone antibiotics. Inhibition of DNA replication by fluoroquinolones is as a result of its binding to DNA gyrase or topoisomerase II, or topoisomerase IV. Resistance to fluoroquinolones in *C. difficile* is associated with particular mutations in gyrA and gyrB, that encode DNA gyrase. Fluoroquinolones are broad-spectrum antibiotics which act on gram-negative and gram-positive bacteria and are able to decrease normal flora in intestine, hence broad use of fluoroquinolones in hospitals fosters spreading of BI/NAP1/027 *C. difficile* strain.[32-36]

HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN TOXIGENICITY AND PATHOGENESIS

Hypervirulent strain BI/NAP1/027 is demonstrated to carry CdtLoc gene locus and encodes CD196 ADP-ribosyltransferase (CDT) or known as binary toxin. Hypervirulent *C. difficile* is able to produce TcdA and TcdB, similar with non-027 ribotypes throughout gene locus of PaLoc. CDT was initially isolated by Popoff et al.[37] The toxin contains two distinct toxin components separately, namely CDTa and CDTb. CDTa, ADP-ribosyltransferase enzyme acts on actin modifying which leads to depolymerization and destruction of actin cytoskeleton inside gut; meanwhile CDTb hitches to gut cells and stimulates CDTa uptake. Destruction by CDT accommodates bacteria adherence and surges Toxin A and B uptake.[38-40] Furthermore, hypervirulent strain contains bp frameshift deletion on TcdC gene, nucleotide 117, and functions as negative regulator of Toxin A and Toxin B. TcdC mutation causes toxins hyperexpression. Warny et al.[58] demonstrated BI/NAP1/027 as able to produce 16 times of Toxin A and 23 times of Toxin B approximately compared to control strain. One research postulated increasing sporulation by hypervirulent strain possibly has association with robust CDI spreading. Nevertheless, previous research demonstrated controverted results in regard to disease severity by hypervirulent strain. A retrospective study by Bauer et al.[41] concluded hypervirulent strain BI/NAP1/027 as associated with declined odds of disease severity ratio (OR): 0.35, 95% confidence interval (CI) 0.13 - 0.93) and did not increase mortality in hospitalized patients (OR: 1.02, 95% CI 0.53 - 1.96), or (OR: 1.16, 95% CI 0.36 - 3.77) of recurrence rate. Meanwhile, some other studies (cohort, case-control, and cross-sectional) did not demonstrate worse prognoses compared to other strains as shown in Table 1.[41]

Table 1. Virulence factors of hypervirulent BI/NAP1/027 *C. difficile* strain[41]

Virulence factors	Mechanism
TcdA or Enterotoxin A (Toxin A)	Destruction of actin within target cells causes infiltration of neutrophil, inflammation, and epithelial cells necrosis.
TcdB or Cytotoxin B (Toxin B)	Destruction of epithelial cells tight junctions causes increasing permeability of vascular, and hemorrhage.
CDTa toxin	Modifies the action with ADP-ribosylation leads to depolymerization of actin and damage of cytoskeleton assists bacteria adherence to epithelial cells of gut.
CDTb toxin	Facilitates CDTa toxin uptake into epithelial lining of gut.
Hypersporulation	Increases bacteria reproduction and spreading.
Mutation of TcD gene	Increases assembly of Toxin A and Toxin B by down-regulation of feedback inhibitor necessitate in diminishing toxin production.

Based on Sirard et al. (2011), even though hypervirulent strain BI/NAP1/027 is able to assemble more toxins, they construct spores in fewer numbers and have not always been associated with severe condition of disease.[42] In contrast, a cohort study by Rao et al.[43] demonstrated association between hypervirulent strain ribotype 027 with severe CDI disease (OR: 1.73, 95% CI 1.03 - 2.89; p = 0.037) and higher mortality rate (OR: 2.02, 95% CI 1.19 - 3.43; p = 0.009) juxtaposed to other ribotypes.[43] Study by See et al.[44] demonstrated similar results by using NAP1 strain, where analysis of multivariate regression depicted increased severity of CDI (OR: 1.66, 95% CI: 1.90 - 2.54) and higher mortality (OR: 2.12, 95% CI: 1.22 - 3.68).[44] Furthermore, a study in Quebec showed the hypervirulent strain ribotype 027 is associated with disease severity, twice more severe frequently in contrast to other strains. Nevertheless, basic reasons of these contradictory results were un-measured confounding factors, setting of study, detection methods of *C. difficile*, size of sample, population of study, and design of study. Therefore, the generalization of the study results has to be examined profoundly. Therefore, given these contrary findings, healthcare workers or providers advised to center their attention on infection treatment based on clinical reasoning and infection marker related to severe infection, as well as episodes of diarrhea, dehydration signs, albumin level, creatinine level, white blood cell (WBC) count, underlying comorbidities, and immunocompromised condition.[45,46]

MECHANISM OF ENDEMIC STRAIN DISPLACEMENT WITH HYPERVIRULENT RIBOTYPE 027 *C. difficile* STRAIN

Transmission of pathogen occur via fecal-oral route with new infections emerge by bacterial spore consumption. *C. difficile* spores are resistance to desiccation and able to persist for about 5 months on hard or solid surface. Merrigan et al.[45] examined spore accumulation in regard to growth cycle of bacteria with results demonstrating that hypervirulent strains have the ability to sporulate faster and causing significant more spore accumulation per total volume compared to non-hypervirulent strains.[45] Increase sporulation rate could elucidate the uncommon soaring recurrence correlated to hypervirulent strains, 4-fold according to Marsh et al.[20,46], as patients tend to transmit the contamination to local surroundings, then re-infect themselves subsequently. Subsequently after dormant bacterial consumption and ingestion, germination of *C. difficile* spore occurs as exposure to combination of bile salts and L-glycine. Vegetative phase of *C. difficile* happen as colonization of host's gastrointestinal tract. Even tough colonization is required to cause the disease, most of infected people prevail asymptomatic. CDI manifestations are arbitrated by production of cytotoxic toxins to large intestine epithelial tissue lead the way of immense colon inflammation and epithelial cell obstruction of the host.[46] Study by Pepin *et al*.[47] and Hubert *et al*.[48] demonstrated doubling rate of severe disease as emergence of ribotype 027 in Canada. Hypervirulent strains associated

with higher rates of symptomatic disease presumed to be result of increased production of toxin or due to intensified variant clostridial toxins.[48] There are three probable mechanisms postulated in accordance to transitions from endemic strains to hypervirulent strains: (1) the more infectious strains are hypervirulent; (2) symptomatic condition with higher rate is caused by hypervirulent strain; (3) outcompete in host's gut can be done by hypervirulent strain.[49]

Throughout stochastic simulation, *C. difficile* hypervirulent strain invasion to human population cherished endemic strain was investigated. Reasoning of some previous models aims to establish infection control strategy in hospital and surroundings. Nevertheless, *C. difficile* has been recognized prominently as a global community pathogen, in preference to just segment of healthcare associated pathogen. In addition, present study has demonstrated major source of infection is community. Nonetheless, in some conditions if community not the primary source, infections suffered by high-risk group in healthcare environment. Underlying cause of difference between endemic strains and hypervirulent strains prevail undetermined regardless of current atypical strains constitute predominant infections in community surroundings. Therefore, the consequence of three distinct pathways of intensified were examined namely increasing pathogen infectiousness, increasing rate of colonization to symptomatic disease, and ability of endemic strains displacement by hypervirulent strains in colonized gut.[49,52] Instinctively, parameters govern these distinct mechanisms have positive correlation to possibility of establishment invading strains in community. Nevertheless, comparison of these parameters' influence on invasion rate and prevalence of resultant equipoise yielded different patterns of epidemiology. In accordance to classic epidemiological comprehension, the rate in which an establish pathogen spreading within susceptible individuals is strongly dependent on coefficient of transmission, as modelled by increasing the hypervirulent strain infectiousness. Simulation demonstrated increased infectious strains tend to establish further, spread robustly, and reach equilibrium to increased prevalence in community. Probability of successful established invasion and current steady circumstances of prevalence has been less influenced by rising colonized percentage on clinical disease experience. If individuals colonized by endemic strains were prone to hypervirulent strains, a weaker correlation was constructed with probability of establishment, and no comprehensible correlation was discerned with equilibrium prevalence outcome. Spreading of novel strain is substantially independent to endemicity of resident strain when gut is colonized by resident strain as uncolonized gut readily.[49-53]

Clinical reports over the past 15 years have demonstrated substantial increase of disease rate in accordance to prominent and robust switch in dominance of *C. difficile* strain. Isolates from PCR-ribotyping in Montreal hospital depicted NAP1/ribotype 027 were not found in 2000 and 2001. Nonetheless, NAP1/ribotype 027 constituted more than 75% isolates collected during the outbreak in 2003 until 2004. Increasing prevalence of CDI disease has corresponded to ribotype 027 dominance in many countries across the world, comprising England with its peak in 2007-2008, European countries, and North America.[49,50] Tying to epidemiological model with present analysis results, apparently hypervirulent strains' ability in displacing endemic strains from readily colonized host's gut is the slightest mechanism facilitates ribotype 027 dominance, resulting in more severe diarrhea and longer recovery period. In spite of investigating a wide range of parameter values, from resistance of colonization to susceptibility counterpart in uncolonized individuals, novel strain is unsuccessful to reproduce heightened level of prevalence associated with emerging hypervirulent strains. It does not invalidate the probability of more competitive hypervirulent strains compared to typical strains within host. However, it still suggests this mechanism is not a pivotal role for successful invasion and hypervirulent strain of ribotype 027 clonal dominance. Importantly, the present study depicted strains' competition inside host's gut is not essential for abrupt prevailing strains switching; all surrogate mechanisms

of hypervirulent distinctly illustrated previous dominant strains are not merely added on invasion of subsequent new strain, yet excluded throughout exploitative competition.[53,54]

ANTIBIOTIC RESISTANCE OF HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN

Investigation of BI/NAP1/027 CDI cases in Panama showed high resistance to several antibiotics: rifampin, ciprofloxacin, levofloxacin, moxifloxacin, and clindamycin, yet remain susceptible to vancomycin and metronidazole. Study tested for several antibiotic susceptibility for ribotype 027 and non-027 ribotype in Canada with findings 92.2% resistance of ribotype 027 to moxifloxacin as opposed to 11.2% of other strains. Correspondingly, ribotype 027 strains (78.2%) showed resistance to ceftriaxone compared to other strains (15.7%). Ribotype 027 was greater than 4-fold higher of minimum inhibitory concentration (MIC) compared to metronidazole (4 μg/ml vs 1 μg/ml). In addition, ribotype 027 strain demonstrated 2-fold higher MIC of fidaxomicin (1 μg/ml vs 2 μg/ml). Nevertheless, resistance for vancomycin and clindamycin was akin both in group of BI/NAP1/027 and other strains. Erythromycin resistance is associated with mutation of methylase genes in ribosome, meanwhile fluoroquinolones resistance is caused by mutation of DNA gyrase. Resistance to fidaxomicin and rifamycin group is linked to methylation of ribonucleic acid (RNA) polymerase. In addition, resistance to linezolid is caused by genes of lincosamide and phenicol. Study in several hospitals in Mexico demonstrated numerous ribotype 027 isolates possesses decreased susceptibility to fidaxomicin even though this antibiotic is unavailable in Mexico and patients had been unexposed to it. Basis for BI/NAP1/027 strain treatment is antibiotics. Presently, specific guidelines of the Infectious Diseases Society of America (IDSA) remain unavailable to BI/NAP1/027 strain.[55-58] Consequently, based on overall CDI treatment guidelines, infection by BI/NAP1/027 strain treatment has been proposed as in Table 2.

Table 2. Suggestive antibiotic treatment for BI/NAP1/027 strain[55]

	1st line treatment	Alternative treatment
Initial non-severe infection	Vancomycin per oral (p.o.), 125 mg, 4 times daily, 10 days	Fidaxomicin p.o., 200 mg, twice per day, 10 days. If unavailable, take metronidazole, 500 mg, three times per day, 10 days
1st non-severe recurrency	Vancomycin p.o., 125 mg, 4 times per day, 10 days	Oral fidaxomicin, 200 mg, twice per day, 10 days
2nd non-severe recurrency	Vancomycin p.o. tapering off: 125 mg, 4 times, 7 until 10 days; 125 mg twice per day, 7 days; 125 mg once daily, 7 days; 125 mg per three days, 14 days	Fidaxomicin p.o., 200 mg, twice per day, 10 days, or transplantation of fecal microbiota
Later non-severe relapse	Transplantation of fecal microbiota	Vancomycin p.o. tapering off with probiotics, fidaxomicin, intravenous immune globulin (IVIG)
Severe disease	Vancomycin p.o. 125 mg, f4 times daily; rise to 500 mg, 4 times per day. This can be applied only if there is no improvement within 24 - 48 hours, or associated side effects, e.g., ileus, renal failure	If patient cannot tolerate vancomycin p.o, fidaxomicin is antibiotic of choice
Ileus	Plus intravenous metronidazole 500 mg, every 8 hours to fidaxomicin or oral vancomycin, consultation to general surgery should be considered	IVIG, intra colonic vancomycin

HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN PREVENTATIVE MEASURES AND CONTROL

BI/NAP1//027 is well-known to cause outbreaks in hospital, and some reports have represented efforts and measures in regard to outbreak control. Muto[56] depicted combined measures to control outbreak in the University of Pittsburgh as a bundle of encompassed education; increment of early detection in regard to CDI requires nurses to make an order of toxin texting, and email notification to alert physicians who treat high risk patients, and establish a management team to evaluate patients tested positive for all *C. difficile* toxins. Expansion of infection control action comprises environmental cleansing with bleach, replacing alcohol hand rubs with water and soap to improve hand hygiene for CDI patients, continuation of contact precautions following diarrhea resolution, audit of infection control, and restriction of targeted antimicrobials.[56] Even though particular effect of each measures was difficult to ascertain, investigators delineated a 78% decrease of CDI incidence and severe CDI proportion. Furthermore, only 13% of *C. difficile* isolates were BI/NAP1/027 strain by 2005, compared to 51% among clinical isolates in 2001.[57-60]

In regard to numerous hospitals outbreak in Quebec, the Canadian government allocated $20 million to upgrade infection control measures in 12 hospitals; whereas in Pittsburgh, various approaches were implemented, comprising domestic measures intensification with thorough environmental cleaning of affected areas and toilets by applying bleach, cleaning all rooms on a subdivision or section if number of nosocomial occurrence exceeded within three weeks; equipment dedication; applying hand washing rather than alcohol rubs; prompt finding of CDI case by daily enhancement of toxin testing frequency in clinical laboratory; prompt empirical treatment and contact precaution practice subsequent to second diarrhea stool; move patients from 4-bed ward if possible; and education to decrease administration of cephalosporin and fluoroquinolone. Consequently, incidence of CDI in these hospitals declined from 22.5 to 12.4 per 1000 admittance as a result of applying these preventative measures, but incidence rates did not reach pre-outbreak extent.[61-65] One hospital in Quebec implemented antimicrobial stewardship when no effectivity was shown in decrease of CDI incidence after executing infection control measures. This unrestrained strategy leaned on education and commentary or assessment from pharmaceutical parties and hence attained administration reduction to 54% of total antibiotic and 23% of targeted antibiotic. Simultaneously, with diminishment in antibiotic consumption, CDI incidence has seen a 60% drop. Targeted antibiotics encompassed second and third generation of cephalosporin, macrolides, clindamycin, and ciprofloxacin. Drop in ciprofloxacin usage has been accompanied by increase. In other places, administration of moxifloxacinwas used as an agent incriminated as high-risk antimicrobial agent.[64,65]

Some factors contribute a significant role of therapy by fluoroquinolones in epidemics era, encompassing enhance resistance of BI/NAP1/027 strains to group of fluoroquinolones, juxtaposed to historical isolates not associated to epidemic isolates, expanded consumption of fluoroquinolones, along with high ascribable risk in regard to fluoroquinolones of this outbreak. However, considering assorted outcomes of certain fluoroquinolones restriction, un-assessed hypothesis that could be a "class effect," subsequently all fluoroquinolones restriction will be a specific potential control course of action in hospital with outbreak caused by BI/NAP1/027 strain. Various measures have been implemented in outbreak control of CDI, especially BI/NAP1/027 which poses a remarkable challenge.[66-68] Coalescence of elderly patients, continual use of antibiotics, contamination of hospital environment with spores are all ideal circumstances of CDI outbreaks, high rate or number of morbidity and mortality. Even though infection control measures, such as environmental cleaning, isolation, and hand hygiene, will persist as keystones course of action to prevent *C. difficile* exposure in hospital, methods to reduce disease

risk following *C. difficile* infection or ingestion have to be reckoned. Nevertheless, decreasing antibiotics use remains absolutely an important approach to reduce CDI risk. These measures have commenced in various hospitals, yet there is still considerable extent to improve antimicrobial stewardship. Methods to neutralize antibiotic disruption of microbiota colonization should be incorporated with biotherapeutic methods, e.g., nontoxigenic *C. difficile* strain administration which has demonstrated to be effective in hamster model. Focus measures also should emphasize on development of vaccine of *C. difficile* to boost immune state of elderly people. Passive immune methods such as monoclonal antibody to enhance immune response to toxin A and B seem to be effective in early stage of clinical trials. Nevertheless, even though current focus is on BI/NAP1/027 *C. difficile* strain, new upcoming epidemic strains are going to emerge in the foreseeable future.[69-72]

CONCLUSIONS

Greater fatality and severity epidemics of CDI have been caused by highly virulent form of *C. difficile* of BI/NAP1/027 that spread widely and robustly over decades. Main risk factors are elderly age, hospitalization, and exposure to antibiotics, e.g., cephalosporins, fluoroquinolones, clindamycin, and penicillin. Virulence factors are TcdA, TcdB, CDTa CDTb; hypervirulence is prone to hypersporulation and mutation of TcD gene leads to toxin hyperexpression. Preventative measures can be done by early cases detection, building a management team to evaluate patient positive with all *C. difficile* toxins, hand hygiene improvement, continuation of contact precautions after diarrhea resolution, audit of infection control, restriction of antimicrobials, and development of a vaccine of *C. difficile*.

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CONFLICT OF INTEREST

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