**INTRODUCTION**

*Clostridium difficile* infection or known as CDI has been recognized as a typical HAIs and contributes to a significant proportion of morbidity and mortality among hospitalized patients with case-fatality rates of multiple infections varying from mild diarrhea to pseudomembranous colitis (PMC), especially in the elderly patients on antibiotic treatment.\(^1\) In addition, the high health costs associated with CDI increase the government's financial burden on health spending. It was noted that half a million infections were associated with CDI in the United States in 2011 with an incidence rate of 8.75 cases/1,000 adult admissions in 2009.\(^1\) In study of Saito et al., 2019 reported the results of *C. difficile* ribotyping without information on the prevalence or incidence of CDI in Japan.\(^2\) The incidence of CDI increased from 1.7/1,000 to 2.7/1,000 adults in Korea and 17.1/10,000 hospitalized patients in Shanghai were associated with CDI.\(^6\) Meanwhile, about 44% and 14% of colitis positive patients were diagnosed positively with *C. difficile* toxin in the Philippines and Malaysia, respectively.\(^7\) A more recent study showed that the prevalence of CDI was 9.2% in Thailand.\(^7\) There are few reports of CDI incidence or prevalence in Indonesia. There were eight types of *C. difficile* strains that appeared in healthy people, while another study showed the prevalence of *C. difficile* (TcdA) was 1.3% in communities and hospitals in Jakarta. Recent reports from Central Java indicate the prevalence of CDI will be 20.6% in 2017.\(^4\)\(^-\)\(^10\)

Several risk factors including advanced age, exposure to antibiotics, and hospitalization are strongly associated with CDI. Regulation of the use of antibiotics in Asian countries is considered poor. There has been a review in Southeast Asian countries describing 47% of pneumonia cases not receiving appropriate antibiotics whereas 54% of diarrheal patients received unnecessary antibiotics, with 40% of antibiotics being prescribed underdose.\(^9\)\(^,\)\(^10\) Elderly individuals on recent antibiotic treatment are at the highest risk for CDI because they lack beneficial gut microbiota and have low immunity due to age and other comorbidities. This group was severely affected and had the highest mortality rate due to CDI with a 2% risk increasing every year after the age of 18. A report describes approximately one in ten deaths from CDI in the elderly in the United States in 2010.\(^10\)\(^,\)\(^11\) There is no data on CDI in the elderly in Indonesia, perhaps because of the lack of supervision in cases of CDI followed by limited laboratory modalities. facilities in hospitals that are capable of diagnosing CDI. In addition, cases of relapse (relapse/reinfection) and death from CDI in the elderly will be higher due to inappropriate treatment.\(^11\)\(^-\)\(^16\)

A severe form of *C. difficile* (CDI) infection caused by a hypervirulent strain identified as North American type 1, class B1 from restriction-endonuclease analysis, ribotype 027 as presented by PCR. The hypervirulent strain caused national CDI outbreaks in European countries, Canada, and the United States. The first reported outbreak of type 027 CDI occurred in Canada where the most severe infection was Quebec in 2005. In the United States, type 027 CDI affects 38 states.\(^17\)\(^-\)\(^19\) Meanwhile, based on the European Centre for Disease Prevention and Control, there...
were infections in 16 countries due to CDI type 027.\textsuperscript{17,18} The hypervirulent toxigenic strain had the toxin genes TcdA and TcdB, an 18-bp deletion in TcdC of the toxin regulatory gene, and a deletion in area 117. This causes a premature stop codon and a frame shift, leading to truncation of the TcdC protein.\textsuperscript{9} The increase in cases of type 027 virulence is associated with more excessive toxin production which is associated with a lack of regulatory control of TcdC.\textsuperscript{20,21} The cohort study estimates that approximately 40% of cases of CDI are community-acquired CDI (CA-CDI). CA-CDI occurs in younger people, less severe symptoms, shorter hospital stay, lower recurrence rate and no deaths have been reported due to CA-CDI. In addition, CDI was also exacerbated by the discovery of hypervirulent strains and the quinolone-resistant antibiotic, gatifloxacin as a substitute for levofloxacin.\textsuperscript{20-23}

Epidemiologically, since 2000, mortality and greater severity of CDI have been attributed to the hypervirulent form of \textit{C. difficile}. The BI/NAP1/027 strain is widespread and vigorous over the past 10 years and has been associated with the CDI epidemic. The most common ribotype in the Middle East is 140, 126, 078, 046, 014, 002, 001, while the more common ribotype in Asia is 018, 017, 014, 002, 001. In North America and Europe, the ribotype 078, 027, 020, 014, 001 has been the utmost strain. Ribotype 027 has been found to have reduced sensitivity to chloramphenicol, imipenem, clindamycin, moxifloxacin, rifampin, and metronidazole.\textsuperscript{23,24} These characteristics have implications for the presentation of more severe disease, higher morbidity and mortality due to antimicrobial resistance compared to other strains. Spores of ribotype 027 grow stronger and easier in hospitals because they are resistant to disinfectants, cleaning and hospital environments. An observational study of diarrheal patients at the Veterans Affairs Medical Center, US showed that approximately 22% were positive for the BI/NAP1/027 strain, its mechanism of pathogenesis, risk factors, currently available treatment options, along with proposed infection prevention and control measures.\textsuperscript{25} Based on those mentioned above, this review aims to further evaluate virulence factors of \textit{C. difficile} as the cause of antibiotic-associated diarrhea (AAD).

\textbf{Clostridium difficile Infection (CDI)}

\textit{Clostridium difficile} is an anaerobic gram-positive bacillus, capable of forming spores and toxins, transmitted to humans by the faecal-oral route. In the United States, \textit{C. difficile} is the most commonly reported pathogen of HAIs. The 2011 surveillance study found 453,000 cases of CDI, 29,000 associated deaths; while about a quarter is obtained from the community.\textsuperscript{6} HAIs by \textit{C. difficile} quadruple the cost of hospitalization leading to an estimated $1.5 billion increase in spending in the US each year. It was recorded that half a million infections were associated with CDI in the United States in 2011 with an incidence rate of 8.75 cases/1,000 adult admissions in 2009.\textsuperscript{8} In Hong Kong, there were more than fifteen thousand cases of CDI from 2006 to 2014 of which most cases were identified as HAIs. A national study in Korea revealed the total incidence of CDI was 2.7 cases/1,000 adult admissions in 2008. CDI is also known for its tendency to relapse among 35% of patients on antibiotic therapy and more than half of CDI recurrences are identified as relapses or recurrences.\textsuperscript{26,27} Due to CDI, about $1.1 billion is used in healthcare costs annually in the US, while around $3 million is associated with healthcare costs in Europe. Compared with reports from countries in Europe and the United States, the prevalence of CDI in Asia is not fully known.\textsuperscript{8} In Korea, a survey of 17 tertiary hospitals from 2004 to 2008 found that the incidence of CDI jumped from 1.7/1,000 to 2.7/1,000 adults. The proportion of community-acquired CDI (CA-CDI) to the total CDI cases in a hospital in Busan was 71.1%, while 59.4% of CDI cases in the emergency department of Seoul Hospital were CA-CDI. Based on a comprehensive study in Shanghai, China from March 2007 to April 2008, the overall incidence of CDI was 17.1/10,000 of the number of hospitalized patients; Mild CDI due to a younger mean age (62.8 years) compared with 63% of patients aged 65 years in a comprehensive European study. In addition, a survey in 13 Asia-Pacific countries showed the proportion of CDI associated with health facilities was 53.6% and CA-CDI was 16.5%. Case reports of CDI in Indonesia are still rare. \textit{C. difficile} was identified in 1.3% of stool samples from Indonesian children. However, these data are insufficient to reflect the global prevalence in Asia. Furthermore, data on the prevalence of CDI in the elderly is still not available to date.\textsuperscript{28,29}

CDI occurs mostly in the elderly, which may be explained by several risk factors including frequent exposure to health care, age-related physiological changes, increased use of antibiotics, changes in gut flora composition, and increased comorbidities.\textsuperscript{8} Frequent health exposures increase the likelihood of contact with an environment contaminated with \textit{C. difficile} endospores and frequent use of antimicrobials. Carrier patients of \textit{C. difficile}, both with and without symptoms, can harbor spores on their skin and transmit them to the environment.\textsuperscript{10,11} Age-related physiological changes also increase the risk of CDI, especially in the immune system. The development and recurrence of CDI has been associated with the ability to produce an immune response, and the ability to produce antibodies to the toxin may influence the progress of colonization and active infection. Aging is accompanied by a decline in the immune system – degeneration of the immune system associated with old age – and has been associated with a decline in the adaptive immune system.\textsuperscript{11,12}

\textit{C. difficile} has the ability to colonize the large intestine, then release exotoxin proteins (TcdA, TcdB) causing colitis in people with risk factors. TcdA and TcdB cause diarrhea associated with \textit{C. difficile}, which inactivates a member of the Rho family, Rho GTPase (guanosine triphosphatase). This is followed by neutrophilic colitis, colonocyte death, functional loss of the gut barrier, and colonization death. CDI disease expression is clinically related to the host immune response and \textit{C. difficile} strains.\textsuperscript{15,19} Dramatic increases in severe CDI in hospitals were initially reported in the early 2000s.\textsuperscript{17,18} Centers for Disease Control and Prevention (CDC) isolates described are restriction endonuclease BI group, North American gel electrophoresis (NAP1), polymerase chain reaction (PCR)
027; therefore as BI/NAP1/027. The characteristics of this strain are high level of resistance to fluoroquinolones, strong toxin production, efficient sporulation rate, and very high mortality compared to less virulent C. difficile. The BI/NAP1/027 strain first originated in North America and Western Europe, but is now spreading to hospitals around the world.\textsuperscript{21,30-32}

Although hospital-acquired CDI is the majority, CA-CDI has increased significantly contributing to 1/3 of new CDI cases. CA-CDI occurs when disease onset begins within 12 weeks in individuals who are not hospitalized or in other health care facilities.\textsuperscript{30,31} CA-CDI may occur in younger patients, who have unclear antibiotic exposure and unknown risk factors. Therefore, the main modes of CA-CDI acquisition are currently being investigated. CA-CDI-related morbidity and mortality remain lower than hospital-acquired CDI. Nonetheless, 40% of CA-CDI patients require hospitalization and recurrence rates are similar to those of HA-CDI.\textsuperscript{31} The effect of gastric acid levels on CDI is still unclear. Theoretically, decreasing stomach acid allows more vegetative organisms to reach the large intestine. However, C. difficile produces spores that are resistant to acidic pH.\textsuperscript{33–35}

\textbf{Virulence Factors of C. difficile}

The main virulence factors of C. difficile are TcdA and TcdB which are encoded by the tcdA and tcdB genes. C. difficile locus has three other genes: tcdR, encoding an alternative sigma factor required for toxin production, tcdC, which encoding an anti-sigma factor, with direct interaction with tcdR and tcdE. PaLoc can be transferred by horizontal pathway to non-pathogenic types.\textsuperscript{36,37} Although PaLoc has several characteristics of mobile genetic elements, it appears immobile within every toxigenic C. difficile types.\textsuperscript{36-41}

TcdA and TcdB are in clostridial glycosylation toxin group, also as well as lethal toxin (TcsL), and also hemorrhagic toxin (TcsH) in Clostridium sordellii, alpha toxin (TcnA) in Clostridium perfringens large cytotoxin TpeL.\textsuperscript{21} The size of the toxin is huge, 250 and 308 kDa, with a high degree of sequence identity. Three main domains: N-terminal domain (active catalytically), translocation domain, and C-terminal (Figure 2).\textsuperscript{41} The toxins are mono glycosyltransferases with similar substrate specificity; both catalyze transfer process of glucose to GTPases in the Rho group (Cdc42, Rho, Rac) in aimed cells of target.\textsuperscript{24,37-47}
Mechanism of Action of TcdA and TcdB

Main steps divided into seven paths: (1) binding of the toxin to host cell surface receptors; (2) toxin internalization via endocytosis mediated by receptor; (3) acidification of endosome; (4) subsequent pore formation; (5) divulse of GTD in endosomes to cytoplasm of host cell; (6) Rho GTPases inactivation by process of glycosylation; and (7) cytopathic and toxin-induced cytotoxic effects.

The intoxication pathway starts from endocytic absorption of toxins via a clathrin- and dynamin-dependent mechanism. Both toxins enter the cell due to their receptor binding surface binding to one or more receptors present on the target cell surface. The receptors are sucrose-isomaltase and glycoprotein 96, on apical membrane of human colonocytes and cytoplasm, leads to inflammatory cascade and facilitate TcdA cytotoxicity.

The process that proceeds from the endosome to the cytosol is called translocation and is determined by the mechanism of pore formation capable of penetrating the endosome membrane and by allowing the release of toxins into the cytosol of the host cell. Once in the cytosol, the toxin undergoes InsP6-dependent autocatalytic cleavage with consequent release of a glucosyltransferase domain (GTD) targeting Rho proteins in the cytosol.

Finally, in autocalytic process, TcdA and TcdB pathways into the host cell cytosol, then TcdA and TcdB glucosylate some elements of the Rho subfamily by transporting the glucose moiety detached from uracil diphosphate glucose (UDP-glucose) to the Thr35/37 residue of the Rho protein. Glycosylation process of Rho protein leading to inactivation therefore glucosyltransferase activity of C. difficile toxin is very important for CDI pathogenesis.

CONCLUSION

C. difficile infection (CDI) has been recognized as a typical cause of HAI's and contributes to a significant proportion of morbidity and mortality among hospitalized patients. Extensive C. difficile culture and toxin examinations are still minimal in many hospitals in various Asian countries. Subsequently, reports of C. difficile in Asia are still rare, while reports of cases of CDI in Indonesia are still rare. Several risk factors including advanced age, antibiotic exposure, and hospitalization are strongly associated with CDI. The main virulence factors of C. difficile are TcdA and TcdB causing cytopathic and toxin-induced cytotoxic effects.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

Wedari NLP conducted literature searches and wrote manuscript. Budayanti NNS conceptualized ideas and framework. Darwinata AE reviewed conceptual framework and final draft of manuscript.

REFERENCES


