Clostridioides difficile infection: An emerging zoonotic disease

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Abstract

Clostridioides difficile is a spore-forming enteric pathogen of public health concern causing a toxin-mediated diarrhea in humans. In several countries, the bacterium has evolved as a hypervirulent, antibiotic-resistant pathogen with concerns for its nosomial and community-assocaited routes for disease transmission. Although the exact routes for community-associated infection have not been substantially elucidated, recent surveillance and genetic diversity analysis of community-borne isolates indicate for the potential spillover of the pathogen amongst the human, animal and environment interfaces. This review article highlights the importance of One Health approach for the control of *C. difficile* infection.

Keywords: *Clostridioides difficile*, zoonosis

Introduction

Clostridioides difficile is a significant human gut pathogen that causes a serious toxin-mediated enteritis in humans (Hookman and Barkin, 2009) . Annually, over 500,000 cases of *C. difficile* infections (CDI) are reported in the United States, which incur about $4.8 billion in healthcare and treatment costs (Lessa *et al.*, 2015). Being attributed as a nosocomial pathogen, *C. difficile* infection (CDI) has been increasingly observed among hospital in-patients undergoing long-term use of antibiotics, proton inhibitors and anti-inflammatory agents, which can lead to gut dysbiosis. Upon accidental ingestion of *C. difficile* spores, the dysbiotic intestinal milieu favorably initiates pathogenetic process of *C. difficile* to establish an intestinal infection (Bartlett, 1992; Kelly and LaMont, 1998; Dial *et al.*, 2006). However, during the last decade or two, evidence suggests that asymptomatic carriers and possibly other unknown sources outside the hospital settings may play a critical role in *C. difficile* transmission (Eyre *et al.*, 2013). For this reason, *C. difficile* has been suggested as a community-associated pathogen (Beaugerie *et al.*, 2003; Hensgens *et al.*,

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2012). More importantly, the increased reports of community-associated *C. difficile* infection (CA-CDI) is observed among young, healthy individuals, who were not previously exposed to antibiotics. In this review, we explain the current epidemiological scenario of CDI as a healthcare associated and community associated infectious disease, the potential of role of food, livestock, pet animals and the environment in contributing to the disease with emphasis to a One Health approach for the control of CDI (Fig. 1).

General taxonomical classification and epidemiological considerations

C. difficile is a gram-positive, anaerobic, spore-forming bacterium that can be found in humans, a wide range of animal species, and the environment (Weese, 2020). Previously, *Clostridioides difficile* was named as *Clostridium difficile* and was classified under the *Clostridium* sensu stricto group. However, a recent reclassification was made since *C.*

difficile was shown to be phylogenetically distant from the rRNA clostridial cluster I and located in cluster XI. The cluster XI has been moved to the family *Peptostreptococcaceae*, and based on the phenotypic, chemotaxonomic and phylogenetic analysis, *C. difficile* was proposed to be renamed as *Clostridioides difficile* (Lawson *et al.*, 2016). Currently, both *Clostridium difficile* and *Clostridioides difficile* are validly used under the provisions of the Prokaryotic Code (Oren and Rupnik, 2018) .

During the late 1970's and 1980's, *C. difficile* was considered as a hospital-borne disease, which is responsible for causing pseudomembranous colitis and diarrhea in individuals who had undergone prolonged antibiotic therapy (Larson *et al.*, 1978; Lance George *et al.*, 1978). The importance of CDI was minimal during those times due to its reduced incidence rate and high recovery rate, since patients responded well to clindamycin, metronidazole or vancomycin administration. Although, recurrent CDI was documented in
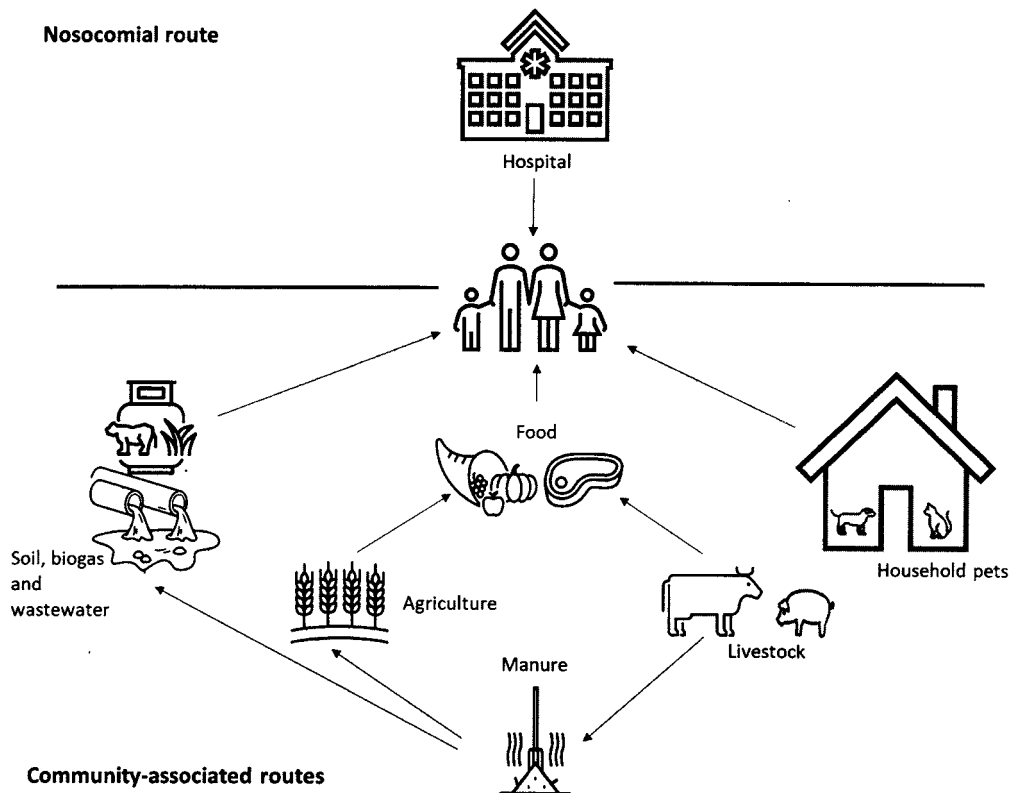

Fig. 1. Sources of C. *difficile* transmission routes to humans

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the past, the condition was easily manageable with only infrequent incidences of the severe disease (Lance George *et al.*, 1978; George, 1988). However, in the early 2000's, a significant epidemiological shift occurred with the emergence of hypervirulent *C. difficile* evincing severe pathological implications and increased antibiotic resistance (He *et al.*, 2013). This fluoroquinolone-resistant hypervirulent strain known as pulsed-field gel electrophoresis type NAP1/restriction endonuclease analysis group BI/PCR ribotype (RT) 027 emerged in North America and later ensued to become a significant burden to the health-care systems worldwide (Spigaglia, 2016). The disease incidence was high mainly in high-income countries and is more commonly seen affecting the elderly, individuals with underlying medical illness, immunocompromised people and long-term hospital in patients requiring prolonged antibiotic therapy (Kim *et al.*, 2011). Although the incidence of CDI has not reduced over the past decade, the prevalence of hypervirulent ribotype 027 has declined in some countries (Turner *et al.*, 2019). Alongside this observation, several community-associated CDI (CA-CDI) cases have also been reported among low-risk individuals without any history of prior hospital admission for prolonged periods and in individuals not exposed to antibiotics (Beaugerie *et al.*, 2003; Lessa *et al.*, 2015; Turner *et al.*, 2019). Moreover, several investigators have documented the emergence of antibiotic resistance in *C. difficile*, especially against fluoroquinolones, clindamycin, erythromycin, metronidazole and vancomycin (Spigaglia, 2016). Consequently, the Centers for Disease Control and Prevention (CDC) in its report on emerging pathogens with antibiotic resistance, categorized *C. difficile* as one of the three urgent threats to public health (CDC, 2019). Early reports of *C. difficile* infection in India dates to the mid-1980s, wherein a prevalence study for *C. difficile* was performed in patients diagnosed with pseudomembranous and antibiotic-associated colitis in North India (Ayyagari *et al.*, 1986). Literature review based on *C. difficile* epidemiology in India reveals that there are only a few reports related to CDI incidence that have been recently published (Joshy *et al.*, 2009; Vishwanath *et al.*, 2013; Hussain *et al.*, 2016; Ghia *et al.*, 2021; Kannambath *et al.*,

2021). The prevalence rate for CDI reported in a tertiary care hospital in Kerala was in the range of 0.06-0.1%, whereas the prevalence rate ranged from 3.4-18.5% across the country (Ghia *et al.*, 2021). With the lack of information related to *C. difficile* prevalence in India, efforts need to be diverted for epidemiological and surveillance studies to monitor prevalence, risk factors and accuracy of *C. difficile* diagnosis for a better understanding of the disease burden in India (Ghia *et al.*, 2021).

In addition, recent CDI epidemiology findings over the past two decades indicate etiological implications for foodborne or zoonotic sources (Knight *et al.*, 2015). Besides food animal sources, *C. difficile* has also been isolated from soil, water, raw vegetables samples and milk (Jobstl *et al.*, 2010; Metcalf *et al.*, 2010; Janezic *et al.*, 2012; Hensgens *et al.*, 2012; Hoover and Rodriguez-Palacios, 2013; Kotila *et al.*, 2013). Although the exact routes of pathogen dissemination are not completely delineated, all reports suggest the likelihood of food and other environmental sources as plausible transmission routes of human CDI especially CA-CDI. Additionally, recent publications indicate that companion animals can act as a likely link for CA-CDI in humans (Hernandez *et al.*, 2020; Rodríguez-Pallares *et al.*, 2022). More information related to the epidemiological routes of healthcare associated and community associated CDI has been described in the subsequent sections.

Pathogenesis for nosocomial *C. difficile* infection

Humans develop CDI primarily by acquiring *C. difficile* spores through the feco-oral route (Hookman and Barkin, 2009). Patients suffering from CDI shed *C. difficile* spores which are resistant structures that can contaminate and survive in the hospital environment such as surfaces and equipments for months, and are extremely resistant to physical and chemical sanitizing agents (Kim *et al.*, 1981; Bettin *et al.*, 1994; Jabbar *et al.*, 2010; Siani *et al.*, 2011). Upon accidental ingestion of spores by susceptible patients, the spores transit through the gastric environment surviving the low pH conditions and eventually reaches the intestine. Susceptible individuals,

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particularly those under prolonged antibiotic treatment will have a dysbiotic gut microflora. Under these circumstances, spores germinate to vegetative cells in the presence of primary bile salts present in the small intestine and the bacterium further establishes and colonizes in the distal gut (colon). In healthy individuals without antibiotic induced gut dysbiosis, distal gut microbiota readily transforms primary bile salts to secondary bile salts. A reduced availability of primary bile salts along with the suppressive action of secondary bile salts on *C. difficile* spore germination and vegetative growth helps to modulate colonization resistance against *C. difficile* by the host's healthy microbiota (Giel *et al.*, 2010; Theriot *et al.*, 2016). In susceptible individuals. upon reaching the colon, the vegetative *C. difficile* colonize and multiply in the intestinal crypts to produce major exotoxins, namely toxin A and B, which are critical virulence factors for CDI (Kuehne *et al.*, 2011). These exotoxins gain entry into the colonic epithelial cells and glycosylate the Rho and Rac GTPases by virtue of their glucosyltransferase domain (GTD). This reaction facilitates destabilization of critical cellular functions such as cytoskeletal disruption eventually leading to tight junction dissociation between colonic epithelial cells and the loss of epithelial integrity (Hunt and Ballard, 2013). As a result, there is an increased intestinal permeability that can favor translocation of bacteria from the gut lumen into deeper tissues (Naaber *et al.*, 1998). Mucosal epithelial damage results in the release of cytokines and chemokines such as IL-1β, IL-8, CXCL-1 and CXCL-2, which promote neutrophil recruitment and activation of resident dendritic cells and macrophage, favoring the release of additional proinflammatory cytokines, including IL-1β, IL-12 and IL-23. Subsequently, innate lymphoid cells are stimulated and release IL-22 and IFN-γ which upregulates macrophage and neutrophil phagocytic activity, production of antimicrobial peptides, reactive oxygen and nitrogen species (RNS and ROS). This process aids further to limit the translocation of other intestinal bacteria. Although inflammatory responses are essential for host survival after CDI, an overactivation of inflammatory responses proceeds to a condition called pseudomembranous colitis, which in advanced

cases, can be detrimental to the host. *C. difficile* toxins in damaged epithelia further promote the release of cytokines such as IL-1, IL-8 and leukotriene-B, which further recruit more neutrophils to the affected region causing additional mucosal injury and focal micro-abscesses and pseudomembrane formation. In adverse conditions, an exaggerated immune response and release of systemically active cytokines, complicated by fluid loss from the resultant severe diarrhea may lead to systemic shock and death (Knight and Surawicz, 2013).

Potential routes for community associated C. difficile infection

Recently, there has been a considerable shift in the epidemiology of CDI (Knight *et al.*, 2015). Whole genome sequencing of isolates from symptomatic patients have shown that clinical *C. difficile* isolates were more diverse and the majority of the cases did not involve any sort of hospital contact. Moreover, reports of *C. difficile* transmission in households, between humans and animals (companion and farm animals), along with the isolation of toxigenic *C. difficile* from retail meat and vegetables suggest a more diverse source for human *C. difficile* acquisitions (Songer *et al.*, 2009; Rodriguez-Palacios *et al.*, 2014; Knetsch *et al.*, 2014; Loo *et al.*, 2016).

Livestock and companion animals as a source of C. difficile

The gastrointestinal tract of mammals (both humans and non-humans) are the preferred habitat for *C. difficile* and, young animals are more frequently colonized than fully-grown animals (Rodriguez *et al.*, 2016). *C. difficile* spores or toxin detection in piglets ranged between 1.4 and 96%, and up to 56% in calves less than three months of age (Rodriguez *et al.*, 2016). Decreased colonization in adult animals could be attributed to the colonization resistance offered by the adult gut microbiota. However, frequent use of antimicrobials, resulted in gut dysbiosis and reduced the colonization resistance resulting in food animals becoming a major source and amplification host for *C. difficile* (Moono *et al.*, 2016). Additionally, use of trehalose in swine production was also considered as a risk

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factor for RT 027 and RT078 carriage in swine (Collins *et al.*, 2018; Turner *et al.*, 2019). The increasing number of *C. difficile* isolation from animals along with reports of RT078 isolation from humans with resistance to tetracycline (an antibiotic widely used in animals) indicates a possible transmission route of these isolates from food animals to humans (Dingle *et al.*, 2019). Foodborne transmission of *C. difficile* can also occur during slaughtering process where shedding of *C. difficile* occur with contamination of carcasses and meat as a result of gut spillage during evisceration or due to the accumulation of spores in the slaughter house (Weese *et al.*, 2011; Olivier Andreoletti, Dorte Lau Baggesen, Declan Bolton, Patrick Butaye *et al.*, 2013). *C. difficile* was isolated from the intestinal contents of up to 28% in pigs, 9.9% in beef cattle and 5% in broiler chickens. Moreover, genomic overlap of RT078 isolates causing human and porcine infections provides evidence for plausible transmission either directly from animals (foodborne transmission) or by an intermediate source (Indra *et al.*, 2009; Hopman *et al.*, 2011; Rodriguez-Palacios *et al.*, 2013; Moloney *et al.*, 2021). For example, feces of colonized or infected animal can act as transmission routes for human infection. Further, core genome analysis of RT078 from diverse sources in 22 countries across four continents revealed extensive clustering of human and animal strains indicating a potential bidirectional spread of *C. difficile* between farm animals and humans (Knetsch *et al.*, 2017). A compararive analysis of molecular characteristics of *C. difficile* isolates from humans and animals in North Eastern region of India also provided evidence that toxigenic isolates from cattle, pigs and poultry could potentially be a source of infection to humans or other animals (Hussain *et al.*, 2016).

However, lack of evidence directly linking food animal transmission and low prevalence of *C. difficile* in animal-derived foods resulted in the search of alternate sources for human infection. The detection of genetically identical and toxigenic *C. difficile* from companion animals, chiefly dog and cat suggests the potential role of household pet as a source for community associated CDI (Hernandez *et al.*, 2020). The close social interaction between companion animals and humans along with the use of similar antibiotics in both species provide a selective advantage and increases the incidental transmission of *C. difficile* in humans (Hernandez *et al.*, 2020). Although *C. difficile* could be normal members of intestinal flora in domestic animals, factors such as antibiotic treatments, changes in diet, poor intestinal motility, pancreatic dysfunction, presence of trypsin inhibitors and parasitic infections can alter the enteric environment of these hosts (Uzal *et al.*, 2016). This results in *C. difficile* overgrowth, which triggers sporulation and toxin secretion (Voth and Ballard, 2005; Uzal *et al.*, 2016). Toxigenic *C. difficile* was isolated from puppies at least once during the first 10 weeks of life (Perrin *et al.*, 1993). However, majority of the colonized dogs were asymptomatic with clinical features and pathogenesis strikingly different from humans. As an example, gut dysbiosis is not a significant feature in companion animals (Uzal *et al.*, 2016; Stone *et al.*, 2019). However, ribotypes shown to produce severe disease in humans such as RT 027, 078, 014/0 and 106 were isolated in dogs and cats and are often found to be antibiotic resistant. Recurrence of RT 106 in humans, a ribotype commonly found in dogs and cats was also reported (Silva *et al.*, 2015; Orden *et al.*, 2017; Rabold *et al.*, 2018). Recently, Rodriguez-Pallares *et al.* (2022) reported the first case confirming the transmission of C. difficile from a dog to a ten-month old female baby.

In India, although reports of *C. difficile* from animals are scanty, the bacterium was recovered from 31.5% of the dogs and 36.5% from pigs in North East India. Out of those positive samples, toxin genes were detected in 55.5% and 33.3% of dog and pig isolates respectively. In another report, toxigenic RT 012, 014 and 046 isolates were recovered from pet dog fecal samples in Assam, India (Hussain *et al.*, 2015; Das *et al.*, 2017). Further, study from Ludhiana, Punjab found *C. difficile* as the second important etiological agent causing diarrhea in canine patients (Sen *et al.*, 2019). Sequence based genotyping methods such as Multilocus sequence typing (MLST) and Multilocus Variable copy Numbers of Tandem Repeats Analysis (MLVA) have shown possible sequence types being shared between animals

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and humans. For example, ST11 which involves the major human hypervirulent RT078 was commonly isolated in food animals (Griffiths *et al.*, 2010).

Food related sources of C. difficile

Considering the obligate anerobic nature of *C. difficile*, contamination of food products with endospores seems to be the possible transmission route of the pathogen in food sources. Although *C. difficile* spores have been detected only at low numbers in veal calf carcass, pork and beef, this might be significant as the spores are resistant to chilling, freezing and recommended cooking temperatures (71°C for over 2 hour) (Flock *et al.*, 2016). Presence of spores in the end products can occur as a result of initial contamination of the raw products, cross contamination of food or due to the production of spores during the processing steps (Rodriguez-Palacios and Lejeune, 2011). When present as spores in those food products, application of heat treatments might enhance the germination of spores and toxin production. However, on the other hand, *C. difficile* spores require a combination of bile salts (taurocholate, glycocholate, cholate and deoxycholate) for germination, and the lack of these ingredients in food matrices might not allow the spore to germinate. Increasing reports of *C. difficile* spore isolation from vacuum packaged and modified atmospheric packaged (MAP) foods also show that changes in food production can influence *C. difficile* prevalence in community settings (Broda *et al.*, 1996; Ghosh *et al.*, 2009; Bouttier *et al.*, 2010; Paredes-Sabja *et al.*, 2014; Atasoy and Gücükoğlu, 2017).

Although a majority of food related sources of *C. difficile* have focused on retail meats (beef, pork, and poultry), *C. difficile* has been isolated from a wide variety of foods, including vegetables (potato, lettuce, pea sprouts, ginger, carrot) and seafoods (clam, salmon, shrimp, mussels) around the world (Pirs *et al.*, 2008; Weese *et al.*, 2010, 2010; Gould and Limbago, 2010; Quesada-Gómez *et al.*, 2013; Rahimi *et al.*, 2014). In retail meats, *C. difficile* was isolated from 1.9 to 6.3% of the samples (von Abercron *et al.*, 2009; Jobstl *et al.*, 2010; Bouttier *et al.*, 2010; De Boer *et al.*, 2011; Rodriguez *et al.*, 2014). However, there has

been a disparity in the prevalence of *C. difficile* in meats from North America and Europe with higher isolation of *C. difficile* from meats in United States and Canada than from Europe (Candel-Pérez *et al.*, 2019). Differences in sampling methods, size of operation, slaughtering practices and types of food examined could have resulted in such differences in prevalence. Prevalence of *C. difficile* isolated from fresh produce (fruits and vegetables) and minimally processed sea food ranged between 2.2 to 7.5% and 3.9 to 49%, respectively (Bakri *et al.*, 2009; Pasquale *et al.*, 2012; Eckert *et al.*, 2013; Troiano *et al.*, 2015). Higher prevalence of toxigenic *C. difficile* in mussels should not be underestimated, as these are eaten raw or partially cooked (Pasquale *et al.*, 2012). Contamination of prepared meals was also reported, and this could have originated from any of the ingredients or as a result of cross-contamination. *C. difficile* RT 017, 027 and 078 associated with community associated CDI were also reported to be isolated from food products (Goorhuis *et al.*, 2008; Weese *et al.*, 2010; Bauer *et al.*, 2011; Rodriguez *et al.*, 2014, 2015). However, no foodborne outbreaks have been reported until today and there have been no epidemiological studies that showed overlap between meat-associated and human infection strains (Turner *et al.*, 2019).

Environment related sources of C. difficile

Toxigenic *C. difficile* has been recovered from environmental sources such as wastewater, river sediments, soil and compost. Studies on *C. difficile* prevalence in European countries showed the greatest positivity rate of *C. difficile* (~100%) in wastewater treatment plant than from any other environment sources (Zidaric *et al.*, 2010; Kotila *et al.*, 2013; Steyer *et al.*, 2015; Moradigaravand *et al.*, 2018; Janezic *et al.*, 2020). Soil samples had unequal distribution of *C. difficile* depending on the soil type. As an example, soil from public environments such as parks, playgrounds, gardens and cultivated field had an overall prevalence rate of 4%, while soil collected from pastures, and paddocks in stables varied between 4 and 11% (Båverud *et al.*, 2003; Orden *et al.*, 2018). Similarly, depending on the substrates for the biogas plant (plant vs animal substrates), *C. difficile* positivity ranged between 4.5 – 58.8%, with

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more positive samples when animal substrates were used in biogas plants (Fröschle *et al.*, 2015; Rodriguez Diaz *et al.*, 2018). Airborne spore transmission of *C. difficile* was also detected from within and around pig production farm with highest positivity recorded in samples collected in pens with neonatal pigs (Keessen *et al.*, 2011). However, the representation of environment as a true source of contamination or is due to the mere consequence of pathogen shedding by carrier or infected animals as a conduit for a specific environmental niche is not yet known. Core genome single nucleotide variant (cgSNV) analysis of *C. difficile* RT 104 of human and porcine isolates revealed interspecies transmission with 42% of human isolates overlapping with at least one animal isolate. However, these clones were recovered months and thousands of kilometers apart across different States of Australia, indicating indirect spread. This study suggests possible interconnected long-range zoonotic and/or anthroponotic transmission that involves recycled waste products such as manure, biosolids and compost which could contaminate the crops resulting in widespread dissemination of *C. difficile*.

Current treatment strategies and novel therapeutic interventions for CDI

Although the prolonged use of antibiotics is known to predispose for CDI, antibiotics ironically remain to be the only approved treatment option for both human and veterinary CDI cases. Currently the recommended antibiotic therapy for CDI includes vancomycin, metronidazole and fidaxomicin. Other drugs not considered as primary choice of treatment include nitazoxanide, rifamixin, ramoplanin, tigecycline, and teicoplanin. The aforesaid antibiotics have been used for cases where severe and adverse effects have been observed with standard therapy, and where it is considered for salvage therapy in cases of fulminant CDI, multiple recurrences, and also where surgical interventions are impossible . Apart from generally used antibiotic agents, other novel antibiotics and adjunctive therapeutics have been developed, which are mostly undergoing human clinical trial evaluation. Some of the novel antibiotics under clinical trials include ACX-362E (synthetic

purine targeting PolC type of polymerase of Gram positive bacteria), DS-2969b (binds the ATP binding site of DNA gyrase), Ridinilazole (inhibits bacterial DNA synthesis), Ramoplanin (a glycolipodepsipeptide antibiotic inhibiting transglycosylases required for peptidoglycan synthesis), DNV3681 (novel fluoroquinolone-oxazolidinone antibiotic), Cadazolid (oxazolidinone antibiotic), Surotomycin (daptomycin derived cyclic lipopeptide) and LFF571 (semisynthetic thiopeptide antibiotic blocking aminoacyl-tRNA delivery during translation) (Pellissery *et al.*, 2019).

Therapeutic adjunctive agents that can aid in restoring the normal gut flora is another treatment modality in C. difficile patients, which include fecal microbiota transplantation (FMT), standardized microbiota replacement therapeutics, probiotic bacteriotherapy and non-toxigenic *C. difficile*. These treatment approaches help to facilitate and modulate for the competitive exclusion of pathogenic *C. difficile* in the gut (Liubakka and Vaughn 2016; Khanna *et al.* 2017; Orenstein *et al.* 2015; Goldenberg *et al.* 2017; Mills *et al.* 2018; Júnior *et al.* 2019; Oliveira *et al.* 2016). Immunization strategies targeting toxin A and B are currently under different phases of clinical trials, however, the first US-FDA approved human monoclonal antibody therapy against toxin B is Bezlotoxumab (ZINPLAVA™) produced by Merck (Peng *et al.*, 2018). Some of the alternative and emerging strategies for CDI therapy include ebselen (glucosyltransferase domain binder), non-absorbable anionic polymers (Tolevamer), and phytochemicals and antimicrobial peptides (Mooyottu *et al.*, 2014, 2017; Furci *et al.*, 2015; Pellissery *et al.*, 2021).

Conclusion

In the past few years, there has been an increasing awareness related to the epidemiological shifts in *C. difficile* prevalence from a nosocomial etiology to a community associated pathogen. Particularly in the United States, community associated infections of *C. difficile* have increased totalling 61% of all CDI cases, suggesting a high possibility of additional sources that can cause CDI in non-hospitalized patients (Fu *et al.*, 2021). The identification

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of genetic relatedness of *C. difficile* isolates derived from humans, animals and food sources plausibly suggests for potential zoonotic and anthroponotic networks for community associated CDI outbreaks (Lim *et al.*, 2020). Although, *C. difficile* control in humans relies on antibiotic stewardship and infection control in healthcare facilities, a critical evaluation from a One Health perspective focusing on potential human, animal and environmental routes for disease transmission will help to understand the epidemiological factors that play a role in *C. difficile* spread and in devising effective control measures.

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