Novel and emerging sources of *Clostridioides difficile* infection

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**Introduction**

*Clostridioides difficile* causes more healthcare-associated infections in the United States than any other pathogen, with an estimated 500,000 infections and 29,000 deaths per year [1]. Although *C. difficile* first emerged as a healthcare-associated pathogen, infections are increasingly acquired in the community [2]. Genomic epidemiology studies have shown that clinical *C. difficile* isolates are more diverse than previously presumed and point toward unidentified environmental reservoirs [3]. An improved understanding of *C. difficile*’s epidemiology is essential for future infection prevention efforts, both within healthcare settings and in the community. Here, we provide an overview of the changing epidemiology of *C. difficile* and current evidence for novel sources of transmission.

**How is *C. difficile* epidemiology changing?**

*C. difficile* first gained attention in the early 2000s, with a rapid increase in healthcare-associated *C. difficile* infection (CDI) driven by the emergence of the hypervirulent North American pulsed-field gel electrophoresis (PFGE) type 1 (NAP1)/027 (PCR ribotype 027) strain [4]. High-level fluoroquinolone resistance likely contributed to the rise of NAP1 incidence in the early 2000s, whereas production of toxin with a higher binding affinity and more rapid cellular entry likely drove the excess mortality rates seen with NAP1 [5,6]. Although total CDI rates have not significantly fallen over the past decade, the prevalence of NAP1 has declined in some regions [4,7]. Over the same time period, an increasing number of CDI cases have been observed among individuals without significant prior hospital contact (referred to as community-acquired CDI, or CA-CDI) [2,8].

For a variety of reasons, the study of CA-CDI is more difficult than healthcare-associated CDI. First, there are few population-based studies of CDI incidence, and the majority of data comes from hospital-based surveillance of CDI among patients admitted to hospitals [1,2,9]. Hospital-based surveillance programs typically follow the National Healthcare Safety Network definitions, which categorize cases of CDI diagnosed within the first 3 days of hospitalization as community-acquired unless the patient was admitted to the same hospital within the preceding 4 weeks. Failure to account for contact with other types of healthcare facilities may misclassify healthcare-associated cases as community-acquired. Robust population-level studies of CDI risk are hindered by difficulties in defining the true population at risk, as well as accurately tracking all potentially relevant outpatient healthcare contacts. One of the major issues with defining populations at risk for CA-CDI is a lack of centralized testing or surveillance. Because patients are able to present to urgent cares, primary care offices, emergency rooms,
and hospitals, often all belonging to different healthcare networks, it is extremely difficult to
determine how many cases are occurring within a particular community.

**What risk factors are associated with CA-CDI?**

Similar to healthcare-associated *C. difficile*, case-control studies consistently associate anti-
biotic receipt with increased risk of CA-CDI [10–12]. Proton pump inhibitors (PPI) have a more
controversial role in CDI risk. Although several epidemiologic studies have linked PPI expo-
sure with healthcare-associated CDI, PPI receipt has not been consistently associated with
increased risk among CA-CDI [13, 14].

Contact with outpatient healthcare facilities (e.g., clinics, urgent cares, dentists) is also com-
mon among patients with CA-CDI [2]. One recent multicenter case-control study conducted
by the Centers for Disease Control (CDC) found that >80% of CA-CDI cases had contact with
an outpatient healthcare facility in the preceding 12 weeks [10]. It remains unclear whether the
healthcare facilities themselves play a direct role in *C. difficile* transmission or if this simply
reflects confounding in which chronically ill patients are both at higher risk of *C. difficile* and
more likely to require frequent care.

Regardless of the exact sources involved, reservoirs for CDI outside of the hospital pose sub-
stantial challenges to prevention efforts given that most current initiatives to prevent CDI
focus on interventions made within hospitals.

**How has molecular epidemiology improved our understanding of
*C. difficile* transmission?**

*C. difficile* persists in the environment, is resistant to many routine disinfectants, and causes
outbreaks among epidemiologically linked patients. For these reasons, it has been presumed
that *C. difficile* is primarily spread from patient to patient through contaminated hands of
healthcare workers, shared equipment, and environmental surfaces. However, current geno-
mic data point toward far more diverse sources of acquisition. A landmark United Kingdom
study using whole-genome sequencing found that 45% of *C. difficile* isolates from hospitalized
patients were genetically distinct from all other cases—consistent with a large and extremely
diverse reservoir for *C. difficile* acquisition [3].

Although many novel sources for *C. difficile* transmission have been proposed, few have
been rigorously confirmed. The sheer diversity of circulating *C. difficile* strains challenges
efforts to untangle patterns of CDI spread. Aside from revealing much greater diversity than
previously anticipated, molecular epidemiologic methods inform many of the potential novel
sources for *C. difficile* transmission, as follows.

**What are the potential novel sources of *C. difficile*?**

**Spread between hospitals and the community**

Several large-scale molecular epidemiologic studies have confirmed overlap between *C. difficile*
strains circulating within the community and among healthcare-acquired cases [9,15]. One
large UK study conducted ribotyping on >700 *C. difficile* isolates from a multicenter network
and found relatively balanced occurrence of most ribotypes between community and health-
care settings [9]. A similar study in Australia arrived at the same conclusion, with nearly 80%
of ribotypes occurring among both community- and healthcare-acquired cases [15]. More
recently, whole-genome sequencing has confirmed the occurrence of genetically related *C. dif-
icile* isolates among both community- and healthcare-acquired cases [16]. The same study
evaluated relevant healthcare contact in the 12 months preceding CDI and found that nearly
all presumed CA-CDI cases had some hospital contact in the preceding year. These data suggest that even distant past healthcare contact may be relevant to subsequent CDI [16]. Despite clear evidence that *C. difficile* transmission can occur between the healthcare and community environments, existing studies are unable to determine the directionality of transmission. Similarly, even though healthcare contact is frequently associated with CA-CDI, it remains unclear if this reflects patients who are actually at elevated risk because of multiple chronic health problems or if contact with healthcare is truly what is driving the risk.

**Role of asymptptomatically colonized hosts**

*C. difficile* carriage rates range from 5.7% to 11.1% among screened inpatients, 7.6% to 24.0% among long-term acute care facility (LTAC) residents, and 16% to 40% among infants (notably, although infants carry *C. difficile* at high rates, they do not develop the disease) [17]. Because asymptomatic carriers can shed *C. difficile* spores in their stool, environmental contamination rates can actually be similar to those with active CDI. Although mathematical modeling studies suggest that implementing contact isolation (placing patients in a single room and requiring healthcare workers to wear gowns and gloves during the patient encounter) for carriers may help to reduce *C. difficile* transmission rates, clinical data supporting the effectiveness and cost-effectiveness of contact isolation for *C. difficile* carriers are lacking [18]. Molecular epidemiologic studies attribute a relatively small minority of transmission events to carriers [3].

**Nonhospital healthcare contacts**

Several large-scale cohort and geospatial studies consistently link nursing home or long-term care facility exposure to increased CDI risk [19]. Given high CDI incidence rates and a high likelihood of readmission to the hospital after developing CDI, expansion of infection prevention efforts to nursing and long-term care facilities is likely necessary for reducing the spread of CDI within healthcare networks.

**Household transmission**

Microbiologic surveys of households with a resident family member recently having had CDI confirm widespread contamination. One large-scale case-contact study found an increased risk for CDI among household contacts of patients with CDI for up to 3 months after occurrence of the index infection [20]. Despite a clearly increased relative risk (RR for spouses of cases: 5.77–9.78), the absolute risk increase for CDI remained small, however (4.71–5.99 per 1,000). Other home surveys found high rates of *C. difficile* contamination across a range of household surfaces even without any known household CDI contacts [21].

**Environmental reservoirs**

*C. difficile* has also been recovered from parks, lawns, soil, beaches, river water, pools, and municipal wastewater, though the relevance of these reservoirs to transmission remains unclear [22,23]. One UK study conducted whole-genome sequencing on regional pairs of clinical and wastewater *C. difficile* isolates, finding both a high degree of genetic diversity overall but also significant overlap between clinical and environmental isolates [22]. Whether environmental contamination is a true source for disease or is simply a consequence of shedding by carriers or infected individuals remains unknown.
Zoonotic potential

*C. difficile* carriage has been well documented for a wide range of animals, including pets, swine, and cattle [24,25]. Ribotype 078 predominates among livestock, and the same ribotype has been associated with community-acquired human cases in some regions. High rates of CDI carriage have also been reported among livestock workers, particularly swine handlers [26]. One Dutch study conducted whole-genome sequencing on *C. difficile* isolates from pigs, human CDI cases, and asymptomatically colonized pig farmers. Just under half of paired *C. difficile* isolates from pig farmers and their respective animals were identical, confirming transmission between animals and humans [27]. Within the same study, several clinical isolates were found to be identical to pig isolates, implicating human–animal transmission in true disease, not just colonization. Subsequent phylogenetic surveys applying whole-genome sequencing to ribotypes 078 and 014 (two isolates well described among both human and animal hosts) found a high degree of co-clustering between human and animal isolates, consistent with frequent bidirectional transmission [28].

Which factors are driving the emergence and transmission of particular zoonotic strains has become a particularly active area of interest, currently focused on feed supplements and agricultural antibiotics. Use of trehalose as a feed supplement in swine has been suggested as a risk factor for *C. difficile* carriage, based in part on genomic and metabolic data. A point mutation in the trehalose repressor of ribotype 027 increases sensitivity to trehalose concentration, whereas ribotype 078 has acquired a cluster of genes that enhance metabolism of trehalose at low concentrations [29]. A recent UK study also found evidence for increasing prevalence of tetracycline resistance among ribotype 078 isolates, suggesting a potential role for the use of agricultural antibiotics in selection of emerging *C. difficile* strains [30].

Foodborne

With high rates of carriage among livestock, it is not surprising that contamination of meat products with *C. difficile* has been reported, including pork, beef, poultry, and processed meats [31,32]. However, no foodborne outbreaks have been described, and molecular surveys show little to no overlap between meat-associated and human disease strains.

Conclusions

Overall, the high degree of diversity among *C. difficile* isolates, increasing evidence of transmission outside of the hospital environment, and multiple potential sources of infection challenge current infection control efforts. Improving our understanding of community-acquired *C. difficile* epidemiology will require detailed prospective collection of wide-ranging exposure-related data on a large scale, coupled with whole-genome sequencing. With the additional issues of widespread outpatient healthcare contact, asymptomatic carriage, and long-term environmental persistence of spores, even the basic distinction between community- versus healthcare-associated CDI may become less relevant with time. Given the challenges posed by current evidence of interspecies transmission and environmental reservoirs of *C. difficile*, future research in *C. difficile* prevention will require an integrative multidisciplinary approach, as exemplified by the OneHealth concept.

References

1. Lessa FC, Mu Y, Barnberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med. 2015; 372(9):825–34. https://doi.org/10.1056/NEJMoai1409913 PMID: 25714160
2. Chitnis AS, Holzbauer SM, Betlour RM, Winston LG, Lyons C, et al. Epidemiology of community-associated Clostridium difficile infection, 2009 through 2011. JAMA internal medicine. 2013; 173(14):1359–67. https://doi.org/10.1001/jamainternalmed.2013.7056 PMID: 23780507

3. Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O’Connor L, et al. Diverse sources of C. difficile infection identified on whole-genome sequencing. The New England journal of medicine. 2013; 369(13):1195–205. https://doi.org/10.1056/NEJMoa1216064 PMID: 24066741

4. Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, et al. The changing epidemiology of Clostridium difficile infections. Clin Microbiol Rev. 2010; 23(3):529–49. https://doi.org/10.1128/CMR.00082-09 PMID: 20610822

5. He M, Miyajima F, Roberts P, Ellison L, Pickard DJ, Martin MJ, et al. Emergence and global spread of epidemic healthcare-associated Clostridium difficile. Nature genetics. 2013; 45(1):109–13. https://doi.org/10.1038/ng.2478 PMID: 23222960

6. Lanis JM, Barua S, Ballard JD. Variations in TcdB activity and the hypervirulence of emerging strains of Clostridium difficile. PLoS Pathog. 2010; 6(8):e1001061. https://doi.org/10.1371/journal.ppat.1001061 PMID: 20808849

7. Katz KC, Golding GR, Choi KB, Pelude L, Amarasingha KR, Taljaard M, et al. The evolving epidemiology of Clostridium difficile infection in Canadian hospitals during a postepidemic period (2009–2015). Cmaj. 2018; 190(25):E758–e65. https://doi.org/10.1503/cmaj.180013 PMID: 29941432

8. Reveles KR, Pugh MJV, Lawson KA, Mortensen EM, Koellner JM, Argamany JR, et al. Shift to community-onset Clostridium difficile infection in the national Veterans Health Administration, 2003–2014. Am J Infect Control. 2018; 46(4):431–5. https://doi.org/10.1016/j.ajic.2017.09.020 PMID: 29126751

9. Fawley WN, Davies KA, Morris T, Parnell P, Howe R, Wilcox MH. Enhanced surveillance of Clostridium difficile infection occurring outside hospital, England, 2011 to 2013. Euro Surveill. 2016; 21(29).

10. Guh AY, Adkins SH, Li Q, Bulens SN, Farley MM, Smith Z, et al. Risk Factors for Community-Associated Clostridium difficile Infection in Adults: A Case-Control Study. Open Forum Infect Dis. 2017; 4(4):ofx171. https://doi.org/10.1093/ofid/ofx171 PMID: 29732377

11. Kutty PK, Woods CW, Sena AC, Benoit SR, Naggi S, Frederick J, et al. Risk factors for and estimated incidence of community-associated Clostridium difficile infection, North Carolina, USA. Emerg Infect Dis. 2010; 16(2):197–204. https://doi.org/10.3201/eid1602.090953 PMID: 2113547

12. Marwick CA, Yu N, Lockhart MC, McGuigan CC, Wuif C, Davey PG, et al. Community-associated Clostridium difficile infection among older people in Tayside, Scotland, is associated with antibiotic exposure and care home residence: cohort study with nested case-control. J Antimicrob Chemother. 2013; 68(12):2927–33. https://doi.org/10.1093/jac/dkt257 PMID: 23825381

13. Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN. A case-control study of community-associated Clostridium difficile infection. J Antimicrob Chemother. 2008; 62(2):388–96. https://doi.org/10.1093/jac/dkn163 PMID: 18434341

14. Naggi S, Miller BA, Zuzak KB, Pence BW, Mayo AJ, Nicholson BP, et al. A case-control study of community-associated Clostridium difficile infection: no role for proton pump inhibitors. Am J Med. 2011; 124(3):276.e1–7.

15. Furuya-Kanamori L, Riley TV, Paterson DL, Foster NF, Huber CA, Hong S, et al. Comparison of Clostridium difficile Ribotypes Circulating in Australian Hospitals and Communities. J Clin Microbiol. 2017; 55(1):216–25. https://doi.org/10.1128/JCM.01779-16 PMID: 27807147

16. Thornton CS, Rubin JE, Grenainger AL, Peirano G, Chiu CY, Pillai DR. Epidemiological and genomic characterization of community-acquired Clostridium difficile infections. BMC Infect Dis. 2018; 18(1):443. https://doi.org/10.1186/s12879-018-3337-9 PMID: 30170546

17. Crobach MJT, Vernon JJ, Loo VG, Kong LY, Pechine S, Wilcox MH, et al. Understanding Clostridium difficile Colonization. Clin Microbiol Rev. 2018; 31(2).

18. Grigoras CA, Zervou FN, Zacharioudakis IM, Siettos CI, Mylonakis E. Isolation of C. difficile Carriers Alone and as Part of a Bundle Approach for the Prevention of Clostridium difficile Infection (CDI): A Mathematical Model Based on Clinical Study Data. PLoS ONE. 2016; 11(6):e0156577. https://doi.org/10.1371/journal.pone.0156577 PMID: 27258068

19. Anderson DJ, Rojas LF, Watson S, Knelson LP, Pruitt S, Lewis SS, et al. Identification of novel risk factors for community-acquired Clostridium difficile infection using spatial statistics and geographic information system analyses. PLoS ONE. 2017; 12(5):e0176285. https://doi.org/10.1371/journal.pone.0176285 PMID: 28510564

20. Pepin J, Gonzales M, Valiquette L. Risk of secondary cases of Clostridium difficile infection among household contacts of index cases. J Infect. 2012; 64(4):387–90. https://doi.org/10.1016/j.jinf.2011.12.011 PMID: 22227466
21. Alam MJ, Anu A, Walk ST, Garey KW. Investigation of potentially pathogenic Clostridium difficile contamination in household environs. Anaerobe. 2014; 27:31–3. https://doi.org/10.1016/j.anaerobe.2014.03.002 PMID: 24657158

22. Moradigaravand D, Gouliouris T, Ludden C, Reuter S, Jamrozy D, Blane B, et al. Genomic survey of Clostridium difficile reservoirs in the East of England implicates environmental contamination of wastewater treatment plants by clinical lineages. Microb Genom. 2018.

23. Alam MJ, Walk ST, Endres BT, Basseres E, Khaleduzzaman M, Amadio J, et al. Community Environmental Contamination of Toxigenic Clostridium difficile. Open Forum Infect Dis. 2017; 4(1):ofx018. https://doi.org/10.1093/ofid/ofx018 PMID: 28480289

24. Rodriguez C, Hakimizadeh E, Vanleysssem R, Taminiau B, Van Broeck J, D’elme M, et al. Clostridium difficile in beef cattle farms, farmers and their environment: Assessing the spread of the bacterium. Vet Microbiol. 2017; 210:183–7. https://doi.org/10.1016/j.vetmic.2017.09.010 PMID: 29103690

25. Collins DA, Selvey LA, Celenza A, Riley TV. Community-associated Clostridium difficile infection in emergency department patients in Western Australia. Anaerobe. 2017; 48:121–5. https://doi.org/10.1016/j.anaerobe.2017.08.008 PMID: 28807622

26. Keessen EC, Harmanus C, Dohmen W, Kuiper EJ, Lipman LJ. Clostridium difficile infection associated with pig farms. Emerg Infect Dis. 2013; 19(6):1032–4. https://doi.org/10.3201/eid1906.121645 PMID: 23735347

27. Knetsch CW, Connor TR, Mutreja A, van Dorp SM, Sanders IM, Browne HP, et al. Whole genome sequencing reveals potential spread of Clostridium difficile between humans and farm animals in the Netherlands, 2002 to 2011. Euro Surveill. 2014; 19(45):20954.

28. Knight DR, Riley TV. Genomic Delineation of Zoonotic Origins of Clostridium difficile. Front Public Health. 2019; 7:164. https://doi.org/10.3389/fpubh.2019.00164 PMID: 31281807

29. Collins J, Robinson C, Danhof H, Knetsch CW, van Leeuwen HC, Lawley TD, et al. Dietary trehalose enhances virulence of epidemic Clostridium difficile. Nature. 2018; 553(7688):291–4. https://doi.org/10.1038/nature25178 PMID: 29310122

30. Dingle KE, Didelot X, Quan TP, Eyre DW, Stuesser N, Marwick CA, et al. A Role for Tetracycline Selection in Recent Evolution of Agriculture-Associated Clostridium difficile PCR Ribotype 078. MBio. 2019; 10(2).

31. Metcalf D, Reid-Smith RJ, Avery BP, Weese JS. Prevalence of Clostridium difficile in retail pork. The Canadian veterinary journal = La revue veterinaire canadienne. 2010; 51(8):873–6. PMID: 21037888

32. Shaughnessy MK, Snider T, Sepulveda R, Boxrud D, Cebelinski E, Jawahir S, et al. Prevalence and Molecular Characteristics of Clostridium difficile in Retail Meats, Food-Producing and Companion Animals, and Humans in Minnesota. J Food Prot. 2018; 81(10):1635–42. https://doi.org/10.4315/0362-028X.JFP-18-104 PMID: 30198756