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MINIREVIEW

Assessing the Burden of *Clostridium difficile* Infection in Low- and Middle-Income Countries

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ABSTRACT In contrast to the significant resources invested in the diagnosis and prevention of *Clostridium difficile* infection (CDI) in resource-rich settings, in resource-limited settings patients with community- and hospital-acquired diarrhea may not routinely be tested for CDI. Is CDI actually less frequent or severe in resource-limited settings, or might we be missing an important opportunity to prevent CDI-related morbidity and mortality (and to promote antibiotic stewardship) in these settings? Here, we review the literature to assess the overall burden of CDI in low- and middle-income countries.

KEYWORDS *C. difficile* infection, CDI, *Clostridioides*, *Clostridium difficile*, global health, low income, middle income, resource limited

In resource-rich settings such as the United States and Europe, significant resources are invested in the diagnosis and prevention of *Clostridium difficile* infection (CDI) (1, 2). In more resource-limited settings, the diagnostic resources are focused elsewhere, and patients with community- and nosocomially acquired diarrhea may not be evaluated for possible CDI. It is notable, given the abundance of efforts to develop diagnostic tests and test platforms to serve populations in resource-limited settings, that the conversation around "global health diagnostics" typically does not include discussion of the diagnosis of CDI. Is this because CDI is actually less frequent or severe in resource-limited settings, or because limited diagnostic resources are simply not being applied to this disease in those settings? What is the actual burden of CDI in resource-limited settings, and if we overlook CDI there, are we missing a potentially important opportunity to prevent morbidity and mortality and to promote antibiotic stewardship?

To find answers to these questions, we conducted a review of recent literature investigating the prevalence and impact of CDI in low-resource settings. To operationalize "low-resource settings," we utilized the World Bank listing of low- and middle-income countries (3) and specifically searched for studies or reviews of studies performed in these countries.

CLINICIANS IN THESE SETTINGS MAY NOT BE CONSIDERING CDI AS A POSSIBLE DIAGNOSIS

In contrast to resource-rich settings such as North America and Europe, where clinicians frequently order testing for CDI (even in patients with very mild diarrhea), investigators considering the burden of CDI in resource-limited settings have pointed out that clinicians in these settings may be much less likely to consider CDI as a diagnosis in patients presenting with community- or hospital-acquired diarrhea. For example, in a 2017 review of CDI burden in Asia (4), the authors stated that "testing remains infrequent, hampered by both a low index of clinical suspicion and the lack of readily available laboratory testing." This point was emphasized by a group working in urban hospitals in Wuhan, China, who stated that "awareness of *C. difficile* and the type

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of infection it could cause was poor and rarely part of the differential diagnosis in patients with diarrhea" (5). Similarly, authors of a study of CDI burden in Nigeria commented on barriers to routine CDI testing related to the perception of low prevalence, in addition to test cost and overall limits to health care system capacity (6). An overall underestimation of CDI burden is not unique to these settings, however; an international survey of clinician awareness of CDI incidence and severity (7) concluded that there was a low overall level of CDI awareness among study participants. However, clinicians in Asia and Europe scored significantly lower on the survey questionnaire than clinicians in North America.

WHEN YOU LOOK FOR CDI, YOU FIND IT, IN RATES THAT ARE SIMILAR TO THOSE IN THE UNITED STATES AND EUROPE

Asia. Borren et al. (4) performed a systematic review and meta-analysis of the literature to define the incidence and impact of CDI in Asia and the Middle East. While acknowledging significant heterogeneity in the studies regarding many key variables, including *C. difficile* testing modalities used to confirm CDI, study populations, and the exact definition of "diarrhea," the authors estimated that the prevalence of confirmed CDI (positive test for toxinogenic *C. difficile* in a symptomatic patient) in patients with diarrhea in East Asia (China, Hong Kong, Japan, South Korea, and Taiwan) was 19.5%, 10.5% in South Asia (India, Malaysia, Pakistan, Singapore, and Thailand), and 11.1% in the Middle East (Iran, Jordan, Kuwait, Lebanon, Qatar, and Turkey). While not all of these countries fall into our working definition of low- or middle-income countries (3), we can still examine these data to assess the geographic distribution of the organism—and overall prevalence of the disease—in these regions. For comparison, a survey of 2011 to 2012 U.S. laboratory *C. difficile* testing data yielded similar overall test positivity rates (i.e., percent positive results in all clinical stool specimens submitted for *C. difficile* testing, many by nucleic acid amplification testing [NAAT]) of 12 to 20% (8). Borren et al. (4) also calculated a pooled CDI incidence rate across Asia of 5.3 cases per 10,000 patient days; for comparison, a European multicenter hospital surveillance study performed in 2008 reported a mean CDI incidence of 4.1 cases per 10,000 patient days (9). Finally, the study by Borren et al. (4) calculated a pooled CDI-related mortality in Asia of 8.9%, while Lessa et al. (10) calculated from 2011 U.S. surveillance data that 9.3% of U.S. patients with health care-associated CDI died within 30 days and estimated that approximately half of those deaths were specifically CDI related. Thus, the cumulative impact of CDI in Asia seems similar to (or perhaps even greater than) the impact of CDI in the United States and Europe.

Individual recent studies from low-resource settings within Asia show a range of prevalence estimates, all similar to the aggregated estimates noted above. As examples, Putsathit et al. (11), using toxigenic culture, found that the prevalence of toxinogenic strains in adult patients with diarrhea in a Bangkok, Thailand, hospital was 9.2%. Similarly, a 2017 study in four hospitals in Indonesia, also applying toxigenic culture to stools of patients with diarrhea, documented a prevalence of toxinogenic strains of 10.9% (12). A 2015 study of inpatients with diarrhea (adults and children >2 years of age) in a tertiary care center in northern India utilizing toxigenic culture found a CDI prevalence of 10.9% (13). A 2015 prevalence study conducted in two urban hospitals in the Wuhan province of China utilizing PCR-based testing of stools from inpatients with diarrhea found a prevalence of 20% in one hospital and 37% in the other (notably, prior to this study, no clinical testing for CDI had been performed in either hospital) (5). Finally, a recent cross-sectional study of hospitalized patients with diarrhea in eastern China documented a CDI prevalence of 10% utilizing *C. difficile* culture and PCR-based toxin gene detection; CDI severity in this cohort ranged from "mild" to "moderate-to-severe" (14).

Sub-Saharan Africa. As observed in reviews of the CDI literature from Asia, a 2016 review by Keeley et al. (15) of CDI studies performed in sub-Saharan Africa noted variable results depending on the laboratory methodology used to detect *C. difficile* and the specific population studied. Two of the ten studies reviewed (both performed prior to 2000) did not detect toxinogenic *C. difficile* at all in the patients studied; however, one study enrolled HIV patients with chronic diarrhea (many of whom were

treated with metronidazole prior to sample collection) (16) and the other utilized suboptimal testing methodology (see below) (17). The other eight studies reviewed concluded that toxinogenic *C. difficile* was prevalent in sub-Saharan Africa. As examples, a 2014 study of outpatients presenting with diarrhea in Zimbabwe found that 8.6% of 268 stool specimens yielded toxinogenic *C. difficile* on culture (18). In a 2013 study in a South African tertiary hospital, Rajabally et al. (19) (using a combination of endoscopy and a toxin A-only enzyme immunoassay [EIA], see below) measured a similar CDI prevalence (9.2%) in adults with diarrhea; 32% of CDI cases were deemed to be community-acquired. The authors also calculated an annual incidence of hospital-acquired CDI of 8.7 cases/10,000 hospitalizations. Twelve percent of the CDI patients underwent colectomy, though CDI patients did not have an increased 30-day mortality rate or rate of intensive care unit (ICU) admission versus controls without CDI.

In their review, Keeley et al. raised the possibility that the combination of unregulated antibiotic use and high HIV prevalence in many parts of this region could lead to a high CDI burden (15). Onwueme et al. (6) performed a study in two urban hospitals in Nigeria, using a rapid EIA for *C. difficile* toxins A and B, and found that the prevalences of CDI in HIV-positive patients with diarrhea in one of the hospitals were 43% and 14% in inpatients and outpatients, respectively (in comparison, the prevalence in a group of predominantly HIV-negative outpatients from the other hospital was ~2%). However, three other studies in the sub-Saharan region found no association between HIV status and an increased prevalence of CDI (reviewed in reference 15).

South America. As in other regions, studies performed in South America vary in terms of diagnostic modalities, study population, and design, and resulting prevalence rates also vary widely. As an example, a 2014 study performed in two Brazilian teaching hospitals concluded that CDI was the cause of 8.3% of cases of nosocomial diarrhea (20); the diagnostic method used in this study was an enzyme-linked immunosorbent assay (ELISA) for toxins A and B in conjunction with positive *C. difficile* culture or positive PCR for the *tcdB* gene. A 2017 study in three hospitals in Colombia utilizing detection of toxins A/B (by EIA) in stools from patients with diarrhea estimated the CDI prevalence rate at 9.7% (21). In contrast, a 2015 descriptive CDI surveillance study in 4 tertiary hospitals in Argentina and Mexico used ELISA for toxins A and B to test stools from hospitalized patients aged ≥40 years who developed diarrhea within 30 days after receiving in-hospital antibiotics and found that 41% of patients with diarrhea tested positive for toxinogenic *C. difficile* (22). Similarly García et al. (23), working in a Peruvian tertiary care setting and utilizing an EIA for toxins A/B, showed that CDI was the cause of 35.2% of cases of nosocomial diarrhea. The calculated hospital CDI incidence was 12.9 cases per 1,000 admissions (23).

C. DIFFICILE STRAIN DISTRIBUTIONS VARY IN RESOURCE-LIMITED SETTINGS

In a 2015 analysis of 2011 U.S. CDI surveillance data, the most common PCR ribotypes identified in both community- and health care-associated cases were 027, 020, and 106 (10); in a European hospital-based survey, the PCR ribotypes 014/020, 001, and 078 were most prevalent (ribotype 027 ranked 6th)(9).

Asia. Collins et al. (24) reviewed ribotyping data available from studies performed in Asia and found that the most prevalent toxinogenic ribotypes in Asia were 017, 018, 014, 002, and 001. In particular, ribotype 017, A^-B^+ strains are widespread in Asia; the lack of toxin A production by these strains demonstrates the importance of using tests that detect both toxins A and B, not only toxin A (now true for most *C. difficile* toxin tests in commercial use). Interestingly, ribotypes 027 and 078, both of which have been associated with outbreaks in North America and Europe (e.g., see references 25 and 26), were only sporadically reported. Similarly, in a recent systematic review and meta-analysis of CDI in Asia, in studies in which molecular typing was performed, the mean proportion of infections due to ribotype 027 was only 0.3%, while the proportion due to ribotype 017 was 14% (4). It is notable that despite the apparently low prevalence of the 027 and 078 "hypervirulent" strains in Asia, the estimates of CDI-related mortality

in Asia are similar to (or higher than) the estimates of CDI-related mortality in the United States (10), as noted above, implicating the 017 strain.

Individual studies published subsequent to the review by Collins et al. (24) have noted similar strain distributions in different regions of Asia. For example, in a 2013 study, Hawkey et al. (27) performed ribotyping on *C. difficile* isolates recovered from inpatients in a hospital in central southern China who had diarrhea and had recently received antibiotics and found that the dominant ribotype was 017 (48% of isolates). In a 2017 study conducted in eastern China over a 3-year period, Jin et al. (14) also found ribotype 017 to be the dominant genotype in hospitalized patients. Putsathit et al. (11) recently performed a similar study in inpatients with diarrhea in a Bangkok, Thailand, hospital and found that of the toxinogenic isolates, the most common ribotypes were 014/020 (A^+B^+) and 017 (A^-B^+). In a 2017 study performed in Indonesia by Collins et al. (12), the most common toxinogenic ribotype was 017 (24% of isolates). In the 2015 study of hospitalized inpatients in Chandigarh, India, by Vaishnavi et al. (13), the ribotypes of the toxinogenic strains yielded in culture were 001, 017, and 106.

Sub-Saharan Africa. Of 32 toxinogenic strains recovered by culture from symptomatic patients in a tertiary hospital in South Africa, 50% were ribotype 017 (toxin A^-B^+) (28); moreover, inpatients harboring this strain had a 30-day mortality rate of 36.3%. Other frequent ribotypes in this cohort (all toxin A^+B^+) were 001 and 015; no 027 strains were recovered (28). It is notable that the same group of investigators, in another study in South Africa, found that some of the ribotype 017 strains had reduced susceptibility to metronidazole (29). Two-thirds of the 017 strains were resistant to moxifloxacin, which is frequently used in this area for the empirical treatment of respiratory tract infections and for multidrug-resistant tuberculosis (MDR-TB) (29).

South America. Balassiano et al. (30), in a 2012 review that assessed the *C. difficile* strain distribution in several Latin American countries, including Argentina, Brazil, Chile, Costa Rica, and Peru, noted that strains recovered from adult patients in these countries included ribotypes 017, 001, 133, 014, 039, 106, 038, 010, 020, 233, and 135; at the time, the hypervirulent strain type 027 had only been described in Costa Rica. The 017 strain was shown to have a particularly high prevalence in a study in Argentina, in which the percentage of CDI cases attributable to this strain was above 75% from 2002 to 2006 in an urban tertiary hospital (31). In the 2015 study of CDI in 4 tertiary hospitals in Argentina and Mexico by Lopardo et al. (22), the predominant strain type recovered was also ribotype 017.

Salazar et al. (21) summarized recent data showing that the 027 strain has now been reported in several additional countries, including Panama, Mexico, Chile and Colombia. The study by Salazar et al. took place in three Colombian tertiary hospitals and showed that the most prevalent ribotypes in CDI patients were ribotypes 591 (28%), 106 (13%), and 002 (7%).

FACTORS THAT MAY HAVE LED TO THE UNDERESTIMATION OF CDI PREVALENCE IN RESOURCE-LIMITED SETTINGS

Interestingly, review of the literature revealed key aspects of study design that might have contributed to overall underestimation of CDI prevalence in the settings discussed here. First, test selection may have impacted conclusions. As an example, a 2000 study that concluded that *C. difficile* did not appear to be an agent of AIDS-related diarrhea in Lusaka, Zambia, used an assay that detected toxin A only (thus missing strains that are A^-B^+, now known to be common in Asia and also in at least parts of Africa [see above]) (17). Similarly, the authors of a review of CDI in Thailand noted that multiple early prevalence studies performed there used toxin A EIA as the only method of detection (32). As a final example, a study performed in Mexico City between 2003 and 2007 utilized a toxin A-only assay to estimate disease prevalence (33).

A second key variable is sample handling. For example, the authors of a prevalence study in Zimbabwe pointed out that in resource-poor countries, refrigeration may not be available for the transport and/or storage of stool specimens prior to testing, which obviously would contribute to degradation of toxin in stool specimens and thus potentially false-negative results of toxin detection tests (but not necessarily culture- or

nucleic acid-based tests) (18). A third factor, noted to be particularly relevant in Latin America, is that anaerobic bacterial culture is not a routine procedure in many laboratories because of cost and lack of supplies (30).

Finally, the easy availability of antibiotics to patients in the community, and the frequency of empirical treatment, can decrease the ability to detect *C. difficile*. In one study of *C. difficile* prevalence in rural Ghana, 35% of all hospitalized patients with diarrhea had taken metronidazole within the 2 weeks prior, predominantly in the community setting (34). Moreover, it is theoretically possible that in these settings, patients assumed to have *Entamoeba histolytica* colitis may actually have CDI. A 2012 study from the Philippines (a region in which amoebiasis is considered endemic) tested the stools of 39 patients with endoscopically proven colitis and found that 43.6% tested positive for *C. difficile* toxins A/B (by ELISA), 25.6% tested positive for *E. histolytica* antigen (by ELISA), and 28.2% tested positive for intestinal parasites (by microscopy); coinfection between *C. difficile* and *E. histolytica* was also observed (35). Given that metronidazole is a first-line treatment both for *E. histolytica* and for CDI, empirical treatment for amoebic colitis may unintentionally be treating CDI.

UNREGULATED ANTIBIOTIC USE IN THESE SETTINGS WOULD BE EXPECTED TO INCREASE THE PREVALENCE OF CDI

Antibiotic misuse is a global problem, and one directly linked to the development of antibiotic resistance. Van Boeckel et al. (36) calculated that global antibiotic consumption spiked 35% between 2000 and 2010, with Brazil, Russia, India, China, and South Africa (all middle-income countries) accounting for 76% of this increase. This report also commented specifically on the inappropriate use of fluoroquinolones and cephalosporins in low-income and middle-income countries and the ability of individuals to purchase these (and other) antibiotics over the counter "without presence of a documented clinical need" (36). Similarly, a 2011 WHO report noted that in developing and transitional countries, approximately half of all acute viral upper respiratory infections and viral diarrheal infections are treated inappropriately with antibiotics (37). Multiple investigators have pointed out the likely link between antibiotic misuse in community and hospital settings in resource-limited areas and CDI burden (e.g., see references 5, 15, 24, and 34). Collins et al. (24) commented that "antibiotics are freely available without prescription in most Asian countries, leading to a misuse in the community" and linked this to concern about community-acquired CDI (24). Galaydick et al. (5), studying nosocomial CDI in Wuhan, China, suggested that even in the hospital setting, antibiotic "usage is loosely regulated." Given the scope of antibiotic misuse in resource-limited settings and the known relationship between antibiotic use and *C. difficile* colonization and disease, it is not a stretch to consider that antibiotic misuse could be contributing substantially to the CDI burden in these settings.

ROUTINE DIAGNOSIS OF CDI COULD AID INFECTION CONTROL EFFORTS IN THESE SETTINGS

A recent study by Allegranzi et al. (38) evaluated the burden of endemic health care-associated infections in developing countries, also utilizing the World Bank classification of low- and middle-income countries. While this study did not specifically evaluate rates of CDI (likely in part due to the lack of data about the prevalence of this particular hospital-acquired infection in these regions), the authors concluded that the pooled prevalence of health care-associated infections (including urinary tract, surgical site, and catheter-related bloodstream infections and ventilator-associated pneumonia) in resource-limited settings was substantially higher than that in the United States and Europe. Importantly, the authors pointed out that hand hygiene is "very often neglected by health care workers in settings with limited resources," and cited an adherence (to hand hygiene protocols) of $<$20% in multiple reports (38). Given the importance of hand hygiene in the control of *C. difficile* transmission, it is reasonable to assume that the transmission rates of *C. difficile* would similarly be higher in resource-limited settings than in resource-rich ones. The authors also cited insufficient microbi-

ological laboratory capacity as a constraint on appropriate surveillance of health care-associated infections in these settings.

As one example of the potential impact of infection control measures on the CDI burden in resource-limited settings, a study by Chaudhry et al. (39) described the implementation of infection control, surveillance, and antibiotic stewardship measures after documenting in a New Delhi, India, hospital in 1999 that 15% of cases of nosocomial diarrhea were due to CDI; over the subsequent 5 years, a decrease in the number of CDI cases was noted even though the number of samples submitted for *C. difficile* testing increased. As examples of the potential depth of the problem, a study in a tertiary hospital in Chandigarh, India, documented (by culture) widespread contamination of clinicians' hands and hospital bedding with *C. difficile* (40), and a study in a tertiary hospital in Lima, Peru, concluded that there was frequent in-hospital transmission of *C. difficile*, particularly between patients sharing a room (23). However, despite the need, a 2016 global review of publicly available national and organizational guidelines for infection control and prevention of CDI identified very few guidance documents from resource-limited settings (41).

CONCLUSION

Our conclusion from review of the literature is that CDI is an important cause of both community-acquired and hospital-acquired diarrhea in resource-limited settings. There is a disconnect between the apparent overall lack of focus on CDI as a possible cause of diarrhea in many of these settings and the prevalence of the disease when it is considered and diagnostic testing consequently is applied. As stated by one group of researchers working in Wuhan, China, "not testing for CDI does not mean that CDI does not exist" (5). Healthcare-associated infections are a critical problem in resource-limited settings, perhaps even more so than in resource-rich settings, and the particularly easy access to antibiotics in the community in these settings compounds the risk of infection with antibiotic-resistant organisms such as *C. difficile*. While additional research to assess CDI severity and outcomes in resource-limited settings would further add to our understanding of the burden of disease, existing data suggest that overall incidence, prevalence, and CDI-related mortality in low-resource settings are similar to those in resource-rich settings, where substantial resources are devoted to education, diagnostic testing, infection control, and antibiotic stewardship in an attempt to reduce the incidence of the disease. In an ideal world, diagnostic resources in low-resource settings would be applied to the diagnosis of CDI; if resource limitations are the predominant barrier, then less expensive diagnostic tests should be developed and made available in these settings. While diagnostic access is being improved, targeted surveillance for CDI (including strain typing and susceptibility testing) in resource-limited settings is prudent to monitor rates of infection and emergence of epidemic strains. Given that strain typing for surveillance purposes is resource intensive, it would need to be performed selectively; however, typing is particularly important given that *C. difficile* strain distributions in low-resource settings have already been shown to differ from those in resource-rich settings (e.g., the high prevalence of ribotype 017 [toxin A^-B^+] strains). A final argument for increasing awareness of this disease—and its potentially severe consequences—in resource-limited settings is that increased awareness could potentially provide a powerful tool to regulate the use of antibiotics in these settings and consequently to limit the development of overall antibiotic resistance.

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