

Pilot surveillance of *Clostridium difficile* infections among patients with diarrhoea in medical facilities of Tbilisi, Georgia

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Ključne riječi

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Background: *Clostridium difficile* infection (CDI) is the most frequent cause of healthcare-associated diarrhoea and is increasingly recognized in the community. The epidemiology of CDI in Georgia is unknown.

Methods: Pilot surveillance for CDI among patients with diarrhoea was conducted in four hospital ICUs, and three outpatient clinics in Tbilisi, Georgia. Demographic, clinical and treatment data of patients with CDI were collected from medical records. A CDI diagnosis was made if the stool sample was positive for *C. difficile* toxin A and/or B by nucleic acid amplification test or enzyme immunoassay, or by culture of a toxin-producing *C. difficile*.

Results: A total of 131 patients with new onset diarrhoeal illness were recruited. Of these, laboratory confirmed CDI was found in 24 % (31/131): 32 % (17/53) of adult and 20 % (11/55) of paediatric ICU patients, 22 % (2/9) adult and 7 % (1/14) of paediatric outpatients. Presenting symptoms were fever (>38 °C) and diarrhoea with a median duration 7 days. Most CDI cases received antibiotics before diagnosis; 94 % of adults and 91 % of children diagnosed in the ICU (median: 12 days); the majority receiving simultaneously two or more antibiotics. In the outpatient settings, 100 % of CDI case-patients received an antibiotic for a median duration of 7 days.

Conclusions: This pilot surveillance demonstrated that *C. difficile* is a common cause of diarrhoea in hospitalized and community patients in Georgia. It highlights the need to improve the knowledge of medical providers regarding the burden of CDI and to establish diagnostic testing at hospital laboratories.

Pilot istraživanje *Clostridium difficile* infekcija u bolesnika s proljevom u zdravstvenim ustanovama u Tbilisiju, Gruzija

Znanstveni rad

Uvod: Infekcije uzrokovane *Clostridium difficile* (CDI) najčešći su uzrok proljeva povezanog sa zdravstvenom skrbi i sve se više prepoznaju u zajednici. Epidemiologija *C. difficile* infekcija u Gruziji nije poznata.

Metode: Pilot istraživanje *C. difficile* infekcija kod bolesnika s proljevom provedeno je u četiri bolničke jedinice za intenzivno liječenje (JIL) i tri ambulantske klinike u Tbilisiju u Gruziji. Podaci o demografskim i kliničkim karakteristikama te liječenju bolesnika s CDI prikupljeni su iz povijesti bolesti. Dijagnoza *C. difficile* infekcije postavljena je ukoliko je uzorak stolice bio pozitivan na *C. difficile* toksin A i/ili B testom amplifikacije nukleinskih kiselina ili metodom enzimskog imunoeseja ili nalazom *C. difficile* koji proizvode toksine u kulturi.

Rezultati: U istraživanje je bio uključen 131 bolesnik s novonastalom dijarealnom bolesti. Od toga je laboratorijski dokazana CDI utvrđena kod 24 % (31/131) bolesnika: u 32 % (17/53) odraslih i 20 % (11/55) djece liječene u JIL-u, 22 % (2/9) odraslih i 7 % (1/14) pedijatrijskih ambulantskih bolesnika. Simptomi su bili povišena tjelesna temperatura (> 38 °C) i proljev s prosječnim trajanjem od 7 dana. Većina bolesnika s CDI primila je antibiotik prije postavljanja dijagnoze; 94 % odraslih i 91 % djece liječene u JIL-u (medijan: 12 dana); većina je istodobno primala dva ili više antibiotika. U ambulantskom okruženju, 100 % bolesnika s CDI primilo je antibiotik u prosječnom trajanju od 7 dana.

Zaključak: Ovo pilot istraživanje pokazalo je da je *C. difficile* čest uzrok proljeva u hospitaliziranih i izvanbolničkih pacijenata u Gruziji. Ono naglašava potrebu za poboljšanjem znanja pružatelja zdravstvenih usluga vezano uz probleme koje nose infekcije uzrokovane *C. difficile* te uspostavom dijagnostičkih testova u bolničkim laboratorijima.

Background

Clostridium difficile is reported to be the most frequent cause of health care-associated diarrhoea in hospitalized patients worldwide leading to significant economic costs on healthcare systems [1 – 10]. *Clostridium difficile* infections (CDI) are also increasingly recognized in the community [11]. In the United States, CDI is responsible annually for 450,000 infections and is associated with 29,000 deaths [12]. In comparison, the epidemiology of CDI in Georgia is not well known due to unfamiliarity of the medical staff with this infection and lack of diagnostic tests. This report describes a pilot surveillance study for CDI in patients with diarrhoea in several hospitals and outpatient offices undertaken to elucidate if *C. difficile* is endemic in Tbilisi, Georgia.

Methods

From March 1, 2014 through February 29, 2016, a surveillance for CDI among patients with diarrhoea was conducted in four hospital intensive care units (ICU) and three outpatient clinics in Tbilisi, Georgia. Two adults and two paediatric hospitals with the largest number of beds in Georgia and the presence of an ICU were enrolled in the study. The adult hospitals had 382 and 259 beds, respectively, including 22 and 14 ICU beds. The paediatric hospitals had 319 and 170 beds, respectively, including 32 and 20 ICU beds. The surveillance was limited to patients admitted to the ICU for ease of recruiting patients and due to the short length of stay in units outside the ICU. Four outpatient general medicine and paediatric offices were also recruited. Patients aged less than 12 months were excluded due to the high rate of *C. difficile* colonization during infancy.

The protocol was approved by the hospital research boards, and signed consents were obtained from patients or patients' family members.

Definitions

Patients with diarrhoea, defined as at least 3 or more unformed stools in 24 hours were diagnosed with CDI if they had a positive laboratory test result for *C. difficile* toxin A and/or B by molecular assay (nucleic acid amplification tests, NAAT) or enzyme immunoassay, or by culture of a toxin-producing *C. difficile* organism [13 – 14].

Patients were classified as community-associated (CA-CDI) or healthcare-associated (HA-CDI) depending on the location of diarrhoea onset and a history of previous exposure to healthcare. CA-CDI was defined as a patient with symptom onset in the community, provided that symptom onset was more than 12 weeks after the last discharge from a healthcare facility [13, 14]. HA-CDI was defined as a patient with symptom onset 48 hours or more after admission to a healthcare facility.

Data collection and analysis

Physicians recruited all patients in ICU with diarrhoea; all patients (or their family members) who were approached in the study gave consent. After informed consent, data was collected from medical records using a case report form. Data abstraction was performed by hospital physicians trained about CDI and this project. Data included patient's age and sex, underlying conditions, admission date, dates of prior hospitalization, specimen date(s), procedures, and clinical signs and symptoms of CDI and exposure to antimicrobial therapy, antacids, and chemotherapy. Data was entered into a database by project epidemiologists and analyzed using a SPSS 20.0 software package.

Specimen collection and testing

Because microbiological laboratories at the participating hospitals and clinics were unable to conduct CDI diagnostic testing, stool specimens were submitted in clean, watertight containers to the Richard Lugar Central Reference Public Health laboratory, Tbilisi, Georgia for testing. Only liquid stool was accepted. Sample collection was conducted as soon as possible after onset of symptoms. If rapid testing was not feasible, specimens were stored at 40 °C (for up to 24 hours of collection) or frozen at –20 °C if the stool could not be processed within 24 hours of collection.

Enzyme-linked immunosorbent assays

In-vitro production of toxins A and B was measured by enzyme-linked immunosorbent assays (ELISA) (tgcBIOMICS GmbH) performed on stool specimens. Because this was one of the first times this type of testing was performed in Georgia and the incidence of CDI is not well-recognized, for further confirmation, specimens positive by ELISA underwent a second step confirmatory PCR test. Those with positives on both tests were considered as true positives in the analysis.

Molecular detection of the *C. difficile* glutamate dehydrogenase (GDH) and toxin genes

Bacterial DNA was isolated from stool samples using Fecal DNA Miniprep kit (Zymo Research). Each sample was tested for the presence of the *C. difficile* glutamate dehydrogenase (GDH) gene (*gluD*), and toxin genes (*tcdA* and *tcdB*) using polymerase chain reaction (PCR). For detection of *gluD*, the primers were *GluD-s* (5'-GTCTTGATGGTTGATGAGTAC-3') and *GluD-as* (5'-TTCC-TAATTTAGCAGCAGCTTC-3') [15]. The *tcdA* gene was detected using in-house primers *Tox-A-s* (5'-TGTTGGAATAGGTGCTGAAG-3') and *Tox-A-ss* (5'-AGATGGAGATGAGAAAAAGTGA-3') whereas the *tcdB* gene was detected using primers *NK104* (5'-GTGTAGCAATGAAAGTCCAAGTTTACGC-3') and *NK105* (5'-CACTTAGCTCTTTGAT TGCTGCACCT-3') [16].

Table 1. Percentage of patients with diarrhoea with laboratory diagnosed *Clostridium difficile* infections, Tbilisi, Georgia, March 2014 – February 2016**Tablica 1.** Postotak bolesnika s proljevom i laboratorijski dokazanom infekcijom uzrokovanom *Clostridium difficile* u Tbilisiju, Gruzija, u razdoblju od ožujka 2014. g. do veljače 2016. g.

| Surveillance Site | Number of patients recruited | Laboratory confirmed CDI No. | % |
|------------------------------------|------------------------------|------------------------------|-----------|
| Hospital ICU subtotals | 108 | 28 | 26 |
| Adult Hospital ICUs | 53 | 17 | 32 |
| Children hospital ICUs | 55 | 11 | 20 |
| Outpatient office subtotals | 23 | 3 | 13 |
| Adult outpatient clinic | 9 | 2 | 22 |
| Paediatric outpatient clinic | 14 | 1 | 7 |
| Overall Total | 131 | 31 | 24 |

Bacteriology and antimicrobial susceptibility testing

Stool specimens from patients with diarrhoea were cultured anaerobically using a selective microbial medium, chromID *C. difficile* agar (bioMerieux, France) [17]. Pretreatment of stools with ethanol-shock (equal volumes of ethanol and faeces mixed for 1 hr before inoculation) was performed to increase the sensitivity of culture [18]. Colonies of *C. difficile* were selected from culture plates based on their typical morphology (ground glass appearance) and confirmed as gram-positive rod-shaped bacilli by Gram staining.

Isolates were tested for antimicrobial susceptibility against metronidazole, clindamycin, moxifloxacin and vancomycin using epsilon test (E-test) strips (bioMerieux, France). A suspension of *C. difficile* equivalent to 1 McFarland turbidity standard was swabbed on Brucella agar plates supplemented with hemin and vitamin K1. An Etest strip was applied onto the agar surface of each plate and incubated in an anaerobic atmosphere at 37 °C. The breakpoints were based on epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, <http://www.eu-cast.org/>). The clinical breakpoints used for metronidazole and vancomycin were ≤ 2 mg/L for susceptible, and > 2 mg/L for resistant. An epidemiological cut-off used to define susceptibility for moxifloxacin was ≤ 4 mg/L, while for clindamycin ≤ 16 mg/L.

Results

Clinical characteristics of CDI patients:

During the 24-month study period, 131 patients with new onset diarrhoeal disease were recruited (Table 1): 53 at an adult ICU, 55 at a children's ICU; 9 at adult outpatient clinics and 14 at paediatric outpatient clinics. Of the

62 adults, 36 were male (58 %), 26 female (42 %); of the 69 children, 36 were male (52 %) and 33 (48 %) were female.

Of the 131 patients, 31 were confirmed as CDI by laboratory testing (see laboratory findings below). Of the 17 adult hospital ICU HA-CDI patients, 6 (35 %) were 60 years or older, the median age was 51 years (range: 26 – 78 years), and the female to male ratio was 0.4. Symptoms of CDI included: fever with a temperature 38 °C in 16 cases (94 %), mild-moderate diarrhoea in 14 cases (82 %) (5 – 10 watery stools a day), and severe diarrhoea in three cases (18 %) (>10 watery stools a day). The median duration of diarrhoea was 7 days (range: 4 – 18 days). None of the patients received *C. difficile* specific treatment before stool specimens were collected, but all were treated with standard CDI regimens once a diagnosis was made, either oral metronidazole or vancomycin. All patients were discharged from the hospitals, none required colectomy and none died.

Of the 11 children hospitalized in the ICU as HA-CDI patients, 9 (82 %) were two years old and older, the median age was 2 years (range: 1 – 11 years), and the female to male ratio was 4.5. Nine patients (82 %) were discharged from hospitals, two died (18 %), both cases of death were not related to CDI. Seven patients (64 %) had mild-moderate diarrhoea (5 – 10 watery stools a day), and four patients (36 %) had severe diarrhoea (>10 watery stools a day), all patients had fever with a temperature >38 °C. The median duration of diarrhoea was 7 days (range: 5 – 10 days).

HA-CDI patients were diagnosed 8 – 43 (median: 17) days after hospital admission and 8 – 35 (median: 12) days after ICU admission. None had onset of diarrhoea within 48 hrs of admission.

Thirteen (77 %) of the adults and 10 (91 %) of the children hospitalized with CDI were intubated. One (6 %) of the children died.

adult and three (27 %) paediatric CDI patients underwent hemodialysis.

Admitting diagnoses of adult hospitalized ICU CDI patients most frequently included brain injury (47 %), lower respiratory disease (12 %), trauma (12), sepsis (6 %), gastrointestinal tract disease (6 %), hysterectomy (6 %), and cardiovascular disease (6 %). Nine (53 %) adults with CDI had underlying comorbid conditions: most frequent were neurological diseases 4 (45 %); one patient (11 %) reported each of the following: diabetes, urinary tract infection, hepatitis C, epilepsy, and Parkinson's disease.

Children hospitalized in the ICU with CDI most frequently had diagnoses of lower respiratory tract diseases (64 %), renal failure (27 %), and cardiovascular diseases (9 %). Five (46 %) children had an underlying comorbid illness; these included anorexia, cancer, cerebral palsy, congenital heart defect and epilepsy.

All three CA-CDI patients presented to the outpatient office for evaluation of prolonged diarrhoea (>5 days). One had an underlying comorbidity: diabetes.

Exposures:

Sixteen (94 %) adults and ten children (91 %) with HA-CDI received antibiotics prior to their diagnosis with a median duration of 12 days. Thirteen (76 %) adults and 6 (54 %) of the children received two or more antibiotics simultaneously. In outpatient settings, 100 % of CA-CDI received an antibiotic for a median duration of 7 days.

The most common antibiotics received before CDI among adult HA-CDI patients were ceftriaxone (53 %) and colistin (47 %). Other antibiotics included meropenem (30 %), piperacillin/tazobactam (29 %) and vancomycin (23 %). Similarly, the most common antibiotics pre-CDI in the paediatric HA-CDI patients were ceftriaxone (45 %) and colistin (45 %) and carbapenems (meropenem and imipenem) (39 %). Other antibiotics included cefepime (27 %) and vancomycin (12 %). Of the three CA-CDI patients, two received ceftriaxone and one ampicillin/sulbactam, both antibiotics were administered intramuscularly.

PPI was used in 59 % of the adult HA-CD patients and in 18 % of the children (18 %). Eleven case-patients received steroids: six (35 %) adults and five (46 %) children. No patient was treated with antidepressants. None of the CA-CDI patients received acid reducers, steroids, or antidepressants in the previous 6 months.

Sixty-five percent of HA-CDI patients had a nasogastric feeding tube.

Potential CDI transmission between ICU patients:

We detected a cluster of three CDI patients at one ICU suggestive of patient to-patient transmission [19]. The patients were all diagnosed within a six day period. Two patients had a high fever (>38 °C), severe diarrhoea (more

than 10 watery stools/day), and severe abdominal pain. One patient had milder symptoms: a low-grade temperature, less frequent diarrhoea (5 – 10 watery stools/day), and minimal abdominal pain. All patients had serious underlying diseases, prolonged hospital stays (>7 days), cephalosporin exposure and nasogastric feeding. The transmission of *C. difficile* was halted by implementing infection control measures (hand hygiene, contact precautions, personal protective equipment, management of fecal hygiene, disinfection of non-critical patient care equipment, and environmental cleaning). No CDI patients were diagnosed during the subsequent 6 month follow-up period.

Laboratory findings

A total of 131 stool samples were collected from patients with new onset diarrhoea. Of these, 31 samples were PCR positive for *gluD*, 30 were positive for both *tcdA* and *tcdB* and one sample was positive for *tcdB* only. PCR results of all 31 samples were in full accordance with EIA results (30 samples were positive for *C. difficile* toxins A and B, and one was only positive for toxin B). Of the positive stool samples, nine (29 %) grew on toxigenic culture. Nine (100 %) *C. difficile* isolates were susceptible to vancomycin, eight (89 %) to moxifloxacin, and five (55 %) to metronidazole. All *C. difficile* isolates were resistant to clindamycin.

Discussion

This is the first report of *Clostridium difficile* infections in Georgia. This pilot surveillance in selected Tbilisi hospitals and outpatient clinics, while having potential limited generalizability, demonstrated that this infection was prevalent in selected hospital and community settings, similar to findings from other countries [20 – 23]. Among our case patients, the infection appeared to be a more common cause of diarrhoea in the adult than in the paediatric population, as 30 % of diarrhoeal stools were positive for *C. difficile* in the adult compared to 17 % in the paediatric population. The diarrhoea was prolonged and in many patients it was severe (>10 bowel movements per day), but none of the patients required colectomy and none died. Diagnosis was often delayed, up to 18 days in one patient, due to unfamiliarity of the medical staff with this infection. The high positivity rate (26 %) in adults with diarrhoea in the ICU, was probably due to recruitment of a high risk population.

Similar to other reports, antibiotic exposure was common; more than 90 % of hospitalized patients (94 % adult and 91 % children) received antibiotics. It is known that antibiotics cause disruption of the protective microbiome in the colon increasing the susceptibility to colonization and toxin production by *C. difficile* [26]. This study, like other studies [23, 25 – 27], showed that third-generation

cephalosporins were the most common antibiotic used before CDI; colistin use was also common. A case-control study will be needed to evaluate the risk of this class of antibiotics for development of CDI. The study also showed that the simultaneous use of two or more antibiotics was common and this is known to be associated with an increased risk of CDI [28]. The use of PPI is also common in adults with HA-CDI, recent meta-analysis has shown that the use of PPI with antibiotics is associated with an increased risk of CDI in hospitalized patients [29].

Several hospitalized patients received tube feeding and there was potential transmission between three patients in one of the ICUs. Acquisition of the organism occurs through the oral-fecal route; transmission is facilitated by contamination of the hospital environment and healthcare workers hands [30]. Reducing the burden of the infection will therefore require: 1) early identification of patients and isolation, 2) appropriate hand hygiene by healthcare workers, 3) environmental cleaning with sporicidal agents and most importantly 4) reducing unnecessary exposure to antibiotics.

This pilot study allowed our laboratory to develop and use various tests such as EIA and PCR for the diagnosis of CDI and to gain experience in trying to culture the organism for antimicrobial sensitivity testing. The ability to culture the organism was less successful than expected and will need to be further evaluated. Future testing will be needed to identify the circulating *C. difficile* strains in Georgia. In addition, continued education of medical staff on this disease will be needed to identify those patients who need treatment and isolation to prevent transmission in the healthcare setting. We are also planning on educating the Georgian clinical microbiological laboratories on methods for the diagnosis of CDI through testing stool by EIA and PCR. NCDC's Lugar laboratory will continue to conduct laboratory testing of CDI suspected patients until testing is implemented by microbiology labs.

Conclusions

While the actual burden of CDI in Georgia has not been determined, this pilot surveillance study suggests that *C. difficile* is a common cause of diarrhoea in hospitalized and community patients with recent exposure to antibiotics in Georgia. Given international experience on its significance as a public health problem, it highlights the need to improve the knowledge of medical providers regarding the burden of this infection. It also supports the need to implement methods for testing by the clinical microbiology laboratories. Future surveillance will focus on identifying the circulating strains beyond Georgia in the Caucasus region.

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Conflict of interest

None.

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