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A prospective study of community-associated *Clostridium difficile* infection in Kuwait: Epidemiology and ribotypes

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ABSTRACT

Clostridium difficile infection (CDI) is increasingly recognized as a significant community acquired pathogen that causes disease in the community. The aim of the study was to investigate prospectively the incidence of community-acquired-CDI (CA-CDI) in Kuwait. Of the 2584 patients with diarrhea, 16 (0.62%) were confirmed cases of CA-CDI. The other notable pathogens were *Salmonella* spp. (0.39%) and *Campylobacter* spp. (0.23%). The mean age was 39 years and the CDI was mild. Exposure to antibiotics in the previous 12 weeks, contact with infant aged <2 years and history of foreign travel was significantly associated with CA-CDI ($P < 0.001$; $P < 0.0001$; $P < 0.002$, respectively). Detected PCR ribotypes were 139 (n = 4) and 014, 056, 070, 097 and 179 (each n = 2). CA-CDI in Kuwait is more likely to occur in younger age and associated with ribotype 139. CA-CDI is not a common problem in Kuwait however extra vigilance must be maintained to detect it in the community even without traditional predisposing factors.

1. Introduction

Clostridium difficile infection (CDI) is an important cause of healthcare-associated (HCA) diarrhea in the developed countries especially in patients receiving antimicrobial agents [1]. Several studies have shown that HCA CDI are associated with prolonged hospitalization, high mortality and adverse outcome [1,2]. It has now been found to be an increasingly important infection in a community setting as well [3], particularly in Europe [4,5], North America [6–8], Australia [9,10], and Asia [11]. In this setting, CDI is often seen in young patients who were previously thought to be at low-risk for developing the infection e.g. healthy peripartum women, children, patients who lack exposure to healthcare settings or antimicrobial agents [12]. Community-acquired CDI (CA-CDI) has been known since 1980s [13], and currently it is estimated that more than one quarter of all CDI are acquired in the community [3]. Factors responsible for emergence of CA-CDI are still obscure. However, CA-CDI may be related to emergence of certain epidemic strains [14], environmental sources like animals, pets, food and water contamination [15], or an increase in the proportion of symptomatic patients or asymptomatic carriers in the community who play a role in spores dispersal leading to increase in person-to-person transmission [9,12,16–18]. In addition, it may be related to the awareness of the general practitioners of CA-CDI or antibiotic pressure. However, more than one third of those patients have no history of such risk factors for CDI [4].

This prospective study was undertaken to establish the incidence of CA-CDI and the PCR ribotypes of the causative *C. difficile*, risk factors, severity of infection and the outcome of CA-CDI in Kuwait.

2. Materials and methods

2.1. Subjects

Patients, 2 years and older, who had diarrhea and who attended their regional polyclinics or adult patients attending outpatient departmental clinics of 6 government general hospitals in Kuwait (Adan, Amiri, Farwaniya, Jahra, Mubarak and Sabah hospitals), who gave no history of hospital admission in the preceding 3 months, were included. Children less than 2 years were excluded because asymptomatic high carrier rates have been reported in children <2 years of age [19,20]. This study was conducted from September 2011 to August, 2013. Ethical approval was obtained for the study from the respective local medical ethics committee in the Ministry

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of Health, Kuwait (No. MTT/2093) and the participants or their legally authorized representatives gave informed consent for their inclusion in the study.

2.2. Case definition

Diarrhea was defined as loose stools, i.e. taking the shape of the container or corresponding to Bristol stool chart types 5–7, plus a stool frequency of three stools in 24 h or fewer consecutive hours or more frequently than is normal for the individual (definition of World Health Organization, http://www.who.int/topics/diarrhoea) [21–23]. According to the European Centre for Disease Prevention and Control [24], an episode of CDI was defined as a patient with diarrhea whose stool takes the shape of the container, and it is positive for *C. difficile* toxin A and/or B without other etiology or endoscopic evidence of pseudomembranous colitis. A recurrence was defined as another episode of CDI, which occurred after stopping the treatment of the initial episode, and re-occurs within 3 weeks following the onset of a previous episode. CA-CDI is defined, in this study, as the onset of symptoms occurring while the patient was outside a healthcare facility and the patient had not been discharged from a healthcare facility within 12 weeks before symptom onset (community onset/community-acquired); or the onset of symptoms occurring within 48 h upon admission to a healthcare facility and the patient had no prior stay in a healthcare facility within the 12 weeks prior to symptom onset (healthcare-facility onset; community-acquired) [24]. For each positive case, his/her bio-details, e.g. age, sex, residence area and the underlying disease were collected. Previous admission to hospital and duration of diarrhea before clinic/hospital visit were carefully recorded. A standard questioner was used to collect the clinical information. Previous antibiotics in the previous 12 weeks and treatment, if any, given for diarrhea were recorded. Recovery, recurrence, mortality and other CDI risk factors were also recorded. Positive results were immediately followed-up by WJ who contacted the patients, General Practitioner and/or treating physicians as well as assessed the risk factors for CA-CDI.

2.3. Control patients

Control group data were collected for assessing the risk factors from groups consisting of patients from whom diarrheal fecal samples were examined for the presence of *C. difficile* and toxin A/B but who were negative for both. They were matched to the cases by age, gender and date (within 2 month of the cases being symptomatic). Three controls were assigned to each case.

2.4. Specimen collection

Single non-repetitive stool samples were sent from the clinics to the referring Hospital Microbiology Laboratories and then transported to the Anaerobe Reference Laboratory (ARL), Department of Microbiology, Faculty of Medicine, Kuwait University by dedicated porters. When samples could not be sent immediately, they were refrigerated or frozen and then sent within the next 24–48 h.

2.5. Microbiological testing

Only diarrheal fecal samples were examined. The samples were tested immediately upon arrival in the ARL by GDH test and for *C. difficile* toxin detection using GeneXpert [25]. All samples were then cultured on Cycloserine Cefoxitin Fructose Agar (CCFA) (Oxoid, Basingstoke, UK). The inoculated agar plates were incubated anaerobically in Anoxomat Anaerobic systems (Mart Microbiology BV, Lichtenvoorde, Holland) [26] for 48 h. Presumptive colonies of *C. difficile* were tested for toxin A/B production using ELISA *C. difficile* TOX-A/B kit (TechLab, Blacksburg, VA, USA) according to the manufacturer's instruction, stored frozen at −70 °C and later typed by PCR-ribotyping. A portion of the fecal samples was also cultured on selective/differential media, MacConkey (Oxoid) and SS agar (Oxoid) for isolation of *Salmonella* spp. and *Shigella* spp. and Campy agar (Oxoid) for *Campylobacter* spp. using standard methods. No viral diagnostic test was done.

2.6. Bacterial identification and PCR-ribotyping

Presumptive *C. difficile* isolates were identified by the presence of typical fluorescence color under UV light and API 20AN (bioMerieux, Marcy l'Etoile, France). *Salmonella* and *Shigella* species were identified by API 20E (bioMerieux), while *Campylobacter* spp. were identified by Gram-stain morphology, oxidase test, motility and API Campy (bioMerieux). Ribotyping of the isolates was done as previously described [27].

2.7. Statistical analysis

The EpiCale 2000, version 1.02 (Brixton Health, Llanidloes, Powys, Wales, UK) was used to compare two proportions of count – with percentages with 95% confidence interval and two sided *P*-value.

3. Results

A total of 2548 consecutive, non-repetitive stool samples were examined from community patients with diarrhea. The age ranged between 2 and 89 years (mean = 39 years). There were 1401 males versus 1147 females with a sex ratio of 1.2:1.

3.1. Incidence of community-acquired C. difficile infection

Stool samples collected for this study were from Adan hospital (797), Amiri (865), Farwaniya (168), Jahra (172), Mubarak (384), and Sabah (162). As shown in Table 1, a total of 14 patients from the above hospitals had stools positive for *C. difficile* toxin with concurrent isolation of toxigenic strains on culture. Two patients had A/B toxins positive in their stools but culture for *C. difficile* was negative. Another 26 patients harbored stool culture-positive *C. difficile* but both stool and isolates were toxin-negative. Four (25%) of these 16 patients with CA-CDI were under the age of 18 years and 10 (62.5%) were between the age 18–65 years and only 2 (12.5%) were over 65 years. Thus, the overall incidence in the community was 0.62% (16/2584). The total incidence rate was 0.049/10,000 over 2 years period i.e. 0.0245/10,000 person years (incidence per year) [28]. Kuwaitis with CA-CDI were 12 (75%) compared to 4 (25%) non-Kuwaitis ($P = 0.00084$; CI 95% [0, 0.02]). Kuwaitis with diarrhea in this study were disproportionately represented in spite the fact that native Kuwaitis are just about 34.5% of the population (1,128,381/3,268,431; Kuwait population in 2012). Among Kuwaitis, the incidence rate was 0.106/10,000 over 2 years period i.e. 0.053/10,000 person years (incidence per a year) [28]. Among non-Kuwaitis, the incidence rate was 0.00187/10,000 over 2 years period i.e. 0.0093/10,000 person years (incidence per a year) [28]. The distribution of the number of fecal sample received and the pathogenic isolates by hospital clinics is shown in Table 1. There were no differences in sex or age distribution by hospital.

3.2. Risk factors

As shown in Table 2, four (25%) of the patients had history of foreign travel compared to only 5 (10.4%) controls; the difference