Prevalence of *Clostridium difficile* infections among hospitalized patients in Amman, Jordan: A Multi-Center Study

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Abstract

Objective: To evaluate the prevalence of *C. difficile* infection (CDI) among hospitalized patients with toxin-positive stools.

Methods: This study is a multicenter study held in Jordan and focused on the prevalence of in-patients with *C. difficile* toxin-positive diarrhea-stools. The study included three hospitals with approximately 750 beds. In-patients charts, laboratory logbooks for in-patients with diarrhea-stool specimens were reviewed. The participating hospitals used a rapid test, which detects fecal *C. difficile* toxins A and B.

Results: 174 stool specimens were reviewed from March 2013 to October 2014, and 170 stool specimens from 168 patients were evaluated. The patients included 102 (60%) males, and 66 (40%) females including seven (10.6%) peripartum females. The patients were classified in the following age groups: neonates ≤ 28 days, infants 29 days - less than one year old ($n = 4$, 2.4%), 1 – 4 years ($n = 3$, 1.8%), and arbitrarily: 5 - 9 years ($n = 3$, 1.8%), 10 – 14 years ($n = 3$, 1.8%), 15 – 40 years ($n = 33$, 19.4%), 41- 64 years, ($n = 53$, 31.2%) and ≥ 65 years were ($n = 71$, 41.8%). Adults and older age groups make up the majority of all patients (92.4%). Comorbidities were highly prevalent among the patients: diabetic ($n = 71$, 41.8%), chronic lung diseases ($n = 25$, 14.7%), solid tumors other than colonic tumors ($n = 12$, 7.1%), immune-suppressive state ($n = 15$, 8.8%), and one patient had colonic tumor. The majority of the patients ($n = 21$) were on more than one class of broad-spectrum antimicrobials. The prevalence of *C. difficile* toxin-positive stools were 14.63/1000 discharged patients, 12.65% of patients (12.96% of stool specimens)

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and 5.0/1000 patient-day. The age-adjusted CDI distribution showed that the rates increased with age and were relatively low in the neonatal period.

Conclusion: The hospital-associated *C. difficile* prevalence showed high rates, particularly in adults and older patients, in patients with a prolonged hospital stay, and comorbidities.

Keywords: *Clostridium difficile* Infection, CDI, Toxin A, Toxin B, Pseu-

domembraneous colitis, Antibiotic Associated Colitis.

Introduction

Clostridium difficile is a Gram-positive endospore-forming bacillus that was discovered as a bowel colonizer and pathogen in 1935. Healthy newborns and infants were found to be colonized with *C. difficile* at rates ranging from 45 to 70%. However, the prevalence of toxigenic strains was found to be as low as 13%. The colonization rates decline as individuals grow older, reaching rates lower than 5% in urban-dwelling healthy adults. However, colonization rates as high as 25-55% have been detected among adult hospitalized patients and nursing home residents [1, 2].

The prevalence and pathogenesis of *Clostridium difficile* infection (CDI) was not particularly high until the early 1970s and 1980s, when it was evident that its rates were rapidly increasing. Over the last two decades, the incidence and prevalence of CDI has increased markedly in hospital environments, where it has acquired greater virulence, exhibited higher recurrence rates, and became less responsive to metronidazole and resulted in higher colectomy rates and mortality. The new strains secrete more toxins and are exemplified by North

American pulsed-field gel electrophoresis type 1 (NAP1), restriction endonuclease analysis type BI (BI), and polymerase chain reaction (PCR) ribotype 027. The increase in CDI prevalence has become so pronounced that it has superseded that of *Staphylococcus* spp. infections in some healthcare systems [3, 4, 5, 6, 7, 8].

The *C. difficile* infection incidence and prevalence increased markedly with the wide spread of antimicrobials, particularly in admitted patients. It was initially associated with clindamycin, although continued experience with antimicrobials showed that other antimicrobials are as likely as clindamycin to be correlated with CDI [9]. The prevalence of CDI in a nosocomial setting has been surging in North American and European hospitals, with estimated numbers of 3.82 per 1000 discharges in 2000 and 8.75 per 1000 discharges in 2008. These increases were particularly prominent among patients at least 65 years of age [10, 11].

Jordan and other Arab countries have few published studies on the prevalence of CDI in hospital settings. Shehabi *et al.* performed a study at Jordan University Hospital in 2000 and found a 9.7% prevalence rate of *C. difficile* isolates or its toxin in stools from patients of all ages with diarrhea through culture and enzyme immunoassay for the detection of *C. difficile* toxin A.

Approximately a decade later, Nasereddin *et al.* demonstrated an escalation in CDI rates in the same university hospital: the prevalence of toxigenic *C. difficile* isolates were found to be 13.7% among adult hospitalized patients, as demonstrated by the presence of toxin genes and in association with diarrhea [12, 13]. A study conducted in Kuwait focused on the PCR ribotyping of environmental and ICU clinical strains. A total of 32 different ribotypes were detected among the clinical isolates, and the predominant toxigenic ribotypes detected were types 097 and 078, which are different from the findings obtained in North America and Europe, which exhibit a dominance of the 027 ribotype [14].

Furthermore, Jordan like most Arab countries lack antimicrobial restriction policies and this lack may be one reason behind the observed high CDI cases, because its prevalence is linked to the misuse of antimicrobials [14, 15]. This study underscores the importance of studying the CDI prevalence in nosocomial settings by shedding light on the limited data regarding this evolving problem in Jordan and other Arab countries.

Materials and Methods

Settings and Design of Study

This study was a prospective multicenter study conducted in Amman, Jordan from March 2013 to October 2014 that included three hospitals with a total of approximately 750 beds. The study was approved by the IRBs in both teaching hospitals and by the medical administrator in the community hospital. The study focused on the prevalence of *C. difficile* toxin-positive diarrhea stools in in-patients excluding community-acquired cases. The patients' charts and the laboratory logbooks for all in-patients with diarrhea stool specimens were reviewed. Several stool tests are available for the detection of *C. difficile* or its toxins [2, 16]. However, the participating hospitals used the "DUO TOXIN

A+B-CHECK-1", an immune-chromatographic rapid test that detects both fecal *C. difficile* toxins A and B (VedaLabs, Parcd'Activités du Londeau BP 181 – 61006, Alençoncedex, France), with excellent sensitivity, specificity, low cost and with rapid turnaround time. (http://www.cdc.gov/HAI/organisms/cdiff/Cdiff_faqs_HCP.html#a7).

Outcome measures

The main outcome measure evaluated in this study is the prevalence of CDI among hospitalized patients, as determined through the analysis of diarrhea stool toxins. This measure was calculated as number of positive CDI patients per 1000 discharged patients (a performance quality indicator in some healthcare systems). The secondary measures are age-adjusted prevalence and the percentage of *C. difficile* toxin-positive stools among clinically suspected patients with diarrhea. The definition for *Clostridium difficile* infections was the following: the presence of symptoms (usually diarrhea; passage of at least three unformed stools in a period of at most 24 h), either a stool test result positive for *C. difficile* toxins or toxigenic *C. difficile* or colonoscopic findings demonstrating pseudomembranous colitis (PMC) and a history of treatment with antimicrobial or antineoplastic agents within the preceding eight weeks. The same previous definition was used to diagnose recurrent CDI, and a response to a *C. difficile* infection-specific therapy was considered suggestive of diagnosis. The following symptoms, signs and laboratory values were utilized in the diagnosis of suspected *C. difficile* infection cases: abdominal pains or cramps, lower-quadrant tenderness, fever (low grade up to 40.6 °C), leukocytosis up to leukemoid reaction, albumin < 2.5 gm/dl (protein-losing enteropathy), ileus or toxic megacolon and colonic perforation [17].

Statistical analysis

The prevalence of *C. difficile* toxins in diarrhea stools was calculated. The denominator is the

number of hospital-discharged patients (positive tests per 1000 hospital-discharged patients). Other calculations base denominator on the length of hospital stay (positive tests per 1000 patient-day) and the percentage of patients with diarrhea stools (positive tests per diarrhea stool specimens). Boxplot analysis was used to clarify the differences between CDI-positive and CDI-negative subgroups within different age categories as a function of the length of hospital stay. Differences with $P \leq 0.05$ were considered statistically significant. One way ANOVA for unmatched groups were calculated for equality of means. SPSS version 18 was used for the data analysis.

Results

A total of 174 patients's tool specimens were reviewed from March 2013 to October 2014; four patients were excluded for being outpatients. One patient experienced four episodes of diarrhea, and one patient experienced two relapse episodes during a prolonged hospital stay. In total, 174 stool specimens from 168 patients were evaluated. The patients included 102 (60%) males and 66 (40%) females including 7 (10.6%) peripartum females. The patients were classified in the following age groups: neonates ≤ 28 days, infants 29 days-less than one year old ($n = 4$, 2.4%), 1 – 4 years ($n = 3$, 1.8%), and arbitrarily: 5 – 9 years ($n = 3$, 1.8%), 10 – 14 years ($n = 3$, 1.8%), 15 – 40 years ($n = 33$, 19.4%), 41- 64 years, ($n = 53$, 31.2%) and ≥ 65 years were ($n = 71$, 41.8%). Adults and older age groups make up the majority of all patients (92.4%). Comorbidities were highly prevalent among the patients: diabetic ($n = 71$, 41.8%), chronic lung diseases ($n = 25$, 14.7%), solid tumors other than colonic tumors ($n = 12$, 7.1%), immune-suppressive state ($n = 15$, 8.8%), and one patient had colonic tumor. The majority of the patients ($n = 21$) were on more than one class of antimicrobial, which

were mostly broad-spectrum agents: carbapenems were prescribed to ($n = 13$, 61.9%) of the patients, quinolones were administered to ($n = 12$, 57.1%) of the patients, cephalosporins was prescribed to ($n = 4$, 19%) of the patients, glycopeptides were prescribed to ($n = 4$, 19%) of the patients, and β -lactam β -lactamases inhibitors were administered to ($n = 7$, 33.3%) of the patients. Details on the prescribed antimicrobials are presented in (Table 1). The prevalence of *C. difficile* toxin-positive stools in the patients were 14.63/1000 discharged patients, 12.65% of patients (12.96% of stool specimens) and 5.0/1000 patient-hospital day (Table 2). The age-adjusted CDI distribution among the categories showed that the rates exhibited a polynomial trend: the rates increased with age and were relatively low during the neonatal period due to a noticeable absence of *C. difficile* infection in patients between 1 and 14 years of age (Figure 1). However, the 15-40 age categories showed the highest positive correlation of CDI with the length of hospital stay, followed by the 41-64 and ≥ 65 categories (Figure 2).

Discussion

In our study, although sample number was modest, the results demonstrated that the *C. difficile* toxin rates in clinical specimens from patients suspected to have CDI according a pre-set definition were not different from the rates reported from other parts of the world. The CDI prevalence was calculated by several denominators in different studies. Prevalence of 14.63/1000 discharged patients and 5/1000 patient-days were in our study (Table 2). In recent studies in Britain, the rates were found to be 2.16/1,000 patient-days [18]. In other studies, higher rates were found among older age groups [10, 11]. The comparison of our current data with other local data published by Shehabi *et al*, who found that 9.7% of diarrhea stools from clinical specimens to be positive for a

Table 1. Characteristics of 168 patients included in the Prevalence of Nosocomial *Clostridium difficile* infections from whom 174 stool specimen were submitted.

Characteristic		Number (%)
Age (years)#: 29 days < 1	≤ 28 days	0.0
		4 (2.4)
	1 - 4	3 (1.8)
	5 - 9	3 (1.8)
	10 - 14	3 (1.8)
	15- 40	33 (19.4)
	41- 64	53 (31.2)
	≥ 65	71 (41.8)
Gender	Male	102 (60)
	Female	66 (40)
	Peripartum females	7/66 (10.6)
Co-Morbidity	Diabetes mellitus	71 (41.8)
	B. asthma/COPD	25 (14.7)
	CHF	26 (15.3)
	CVA	26 (15.3)
	Sepsis/Bacteremia	24 (14.1)
	Solid tumour	12 (7.1)
	Immunosuppression	10 (5.9)
	Hematological Malignancy	5 (2.9)
	Colonic tumour	2 (1.2)
	Others*	101(55.4)
Antimicrobials** used by <i>C. difficile</i> infected patients within 8 weeks of presentation		<i>C. difficile</i> -toxin positive stools/total positive counts (N = 21, and%)&
	Quinolones	12 (57.1)
	Cephalosporines	4 (19)
	Carbapenems	13 (61.9)
	Penicillins	1 (4.8)
	β-lactams, β-lactamsase inhibitor	7 (33.3)
	Glycopeptides	6 (28.6)
	Colistin	4(19)
	Tigecycline	2 (9.5)
		2 (9.5)

&: Aminoglycosides, clindamycin, sulpha, linezolid, antifungal and antineoplastic each equals 0.0

*Other Comorbidities/presenting diagnoses: mostly coronary heart diseases and hypertension. Trauma (7), CAUTI/UTI (1), CLABSI 1, SSTI (2), Abdominal sepsis

(3), Meningitis (1) and renal Failure.

** Multivariate testes of in between anti-infective agents for causation of CDI (P > .05)

#Total numbers in this box (174) refers to the number of stools specimen for the 168 patients.

Table 2. The prevalence of CDI among suspected hospitalized 168 patients with 174 stool specimens**, the estimations were based on detection of toxins A, B or both in stools[§] specimen.

<i>C. difficile</i> -Toxin positive stools per:	Rates
1000 Discharged Patients*	14.63
Patients with diarrhoea	12.65%
Submitted stool specimens	12.96%
1000 Patient-days	5.0

*Outcome measure.

CDI: *Clostridium difficile* infection

[§] 95.3% were diarrhoea stools according to the definition in the text (Materials and methods).

**One patient with prolonged hospital stay submitted 4 specimens and one submitted two specimens.

C. difficile toxin, revealed that our data paralleled the denominator and methodology of the previous study. The present study showed that 12.96% of suspected patients with diarrhea were positive, showing an increase over the last 12 years of 3.26% ($P < 0.0001$) both patient populations originated from the same geographic area [12]. This finding demonstrates that rates in Jordan are increased over time at a rate similar to that observed worldwide [20]. Though *C. difficile* infection has exhibited an increase in the last few years, this may still be considered an underestimation due to limitations of hospital surveillance, as some of these cases are discharged to community without being captured by healthcare systems [21].

The prevalence of nosocomial CDI was demonstrated to be related to the time of hospital stay. Earlier studies showed that this prevalence is associated with the length of ICU stay [22],

Figure 1. *C. difficile* infection distribution ratios (*C. difficile*-Toxin positive-patients) according to different age groups, with polynomial trend line. CDI: *Clostridium difficile* infection. *C. difficile* Toxin-positive patients (toxin-positive age groups were included in analysis): $F = 0.61$, $P = 0.64$, by ANOVA. Total *C. difficile* infection ($N = 21$); age group ≥ 65 ($n = 8$), 41 – 64 ($n = 9$), 15 – 40 ($n = 3$) and ($n = 1$) positive *C. difficile* infection in 29 days – 1 year group.

Figure 2. Boxplot representation of the patients with *C. difficile* infection distributed according to length of hospital stay (Y-axis), and different age groups (X-axis) clustered in pairs as positive and negative *C. difficile*-toxin. CDI: *Clostridium difficile* infection. Open circle: Moderate outliers. Stars: Extreme outliers. $P > 0.05$ for within age groups, and among age groups, significance was analyzed by ANOVA.

because nosocomial transmission often occurs in this setting due to the occasional break in infection control practices and the increased prescription of antimicrobials [10,23].

The patients in this study exhibited high rates of co-morbidities, and the results were skewed toward adults and older population (92.4%) and included a minority of pediatric patients. The results showed a relative increase in the neonatal period (**Figure 2**).

Co-morbidities were prevalent and 38.2% of the patients were on steroids for different reasons. In addition, many of the patients were suffering from chronic lung diseases, were on immunosuppressive therapy and had solid tumors. Moreover, 41.8% of the patients were diabetic, a patient population that is expected to stay longer in the hospital, which places them at a higher risk for nosocomial

C. difficile infection acquisition, not including the prolongation of their hospital stay due to *C. difficile* infection [22].

In conclusion, our study employed an immunochromatographic rapid assay test to demonstrate that *C. difficile* infection prevalence in Jordan is not different from that found in other parts of the world. The development of other methods for *Clostridium difficile* identification, such as PFGE, restriction endonuclease analysis, EIA and PCR, is needed to help clinicians avoid overlooking cases that may escape detection through the immunochromatographic rapid assay test [24], and novel surveillance methodologies to include unaccounted discharged patients [21]. Of note, none of our patients had a perforated colon, severe toxemia, bleeding or a toxic megacolon. This study focused

on the hospital-associated CDI. Data on community-associated CDI is sparse and hope fully in near future a plan for a study by this group will be implemented to assess the prevalence of the community-associated CDI, which will increase the detection of the outpatient CDI without undue delay. Specially, CDI, a potentially serious illness, may occur in a population without the traditional risk factors but share the degree of severity [25, 26].

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