Prevalence of *Clostridium difficile*-associated diarrhoea among hospitalized Jordanian patients

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معدلات انتشار الإسهال المرتبط بالمِطثِّية العسرة في المرضى الأردنيين الذين أدخلوا المستشفى

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الخلاصة: قمنا باستقصاء عينات من براز 400 مريض بمستشفى الجامعة الأردنية (300 منهم مصابون بإسهال سريري و100 من الشواهد غير المصابين بالإسهال) للتأكد من وجود المطثية العسرة *Clostridium difficile* أو ذيفانها. وتبيَّن لنا انتشار المطثية العسرة أو ذيفانها في براز 9.7% من مرضى الإسهال. أما معدل انتشار مسبِّبات المرض المعوية المحتملة الأخرى، مثل أنواع السلمونيلة *Salmonella* (2.3%) وأنواع الشيغيلة *Shigella* (1%) والمتحوِّلة الحالة للنسج *E. histolytica* (2.7%) فكان أقل بصورة ملموسة. وبلغ معدل انتشار المطثية العسرة أو ذيفانها في الشواهد 3%. وتم اكتشاف وجود الذيفان "أ" في 93.1% من حالات الإسهال المرتبطة بالمطثية العسرة، وذلك باستخدام المقايسة المناعية المرتبطة بالإنزيم. وتدل دراستنا على أن أغلب حالات الإسهال المرتبط بالمطثية العسرة تُلاحظ بين المرضى الأردنيين الذين أدخلوا المستشفى، من الذين تبلغ أعمارهم 50 عاماً فأكثر، مع ارتباط هذه الحالات بالعلاج بمضادات الجراثيم.

ABSTRACT We investigated stool specimens of 400 patients at Jordan University Hospital (300 patients with clinical diarrhoea and 100 controls without diarrhoea) for the presence of *Clostridium difficile* or its toxin. We found a 9.7% prevalence rate of *C. difficile* or its toxin in stools of patients with diarrhoea. The prevalence of other potential enteric pathogens, such as *Salmonella* spp. (2.3%), *Shigella* spp. (1.0%) and *Entamoeba histolytica* (2.7%), was significantly less. Prevalence of *C. difficile* or its toxin in controls was 3.0%. Toxin A was detected in 93.1% of *C. difficile*-associated diarrhoea cases using an enzyme immunoassay. Our study indicates that *C. difficile*-associated diarrhoea is mostly observed among hospitalized patients aged ≥ 50 years, in association with antimicrobial treatment.

Prévalence de la diarrhée à *Clostridium difficile* chez des patients jordaniens hospitalisés

RESUME Nous avons examiné les échantillons de selles de 400 patients à l'Hôpital universitaire jordanien (300 patients souffrant de diarrhée clinique et 100 témoins n'ayant pas de diarrhée) cherchant à déceler la présence de *Clostridium difficile* ou de sa toxine. Nous avons trouvé un taux de prévalence de 9,7 % pour *C. difficile* ou sa toxine dans les selles des patients atteints de diarrhée. La prévalence d'autres agents pathogènes entériques potentiels, tels que *Salmonella* spp. (2,3 %), *Shigella* spp. (1,0 %) et *Entamoeba histolytica* (2,7 %), était considérablement moindre. La prévalence de *C. difficile* ou de sa toxine chez les témoins était de 3,0 %. La toxine A a été détectée dans 93,1 % des cas de diarrhée à *C. difficile* en utilisant un dosage par une méthode immunoenzymatique. Notre étude indique que l'on observe la diarrhée à *C. difficile* principalement chez les patients hospitalisés d'âge ≥ 50 ans, en association avec le traitement par antimicrobiens.

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Introduction

Clostridium difficile frequently colonizes the human large intestine when the normal colonic flora is disturbed by antibiotic therapy. The result of colonization may be asymptomatic, or it may lead to illness, ranging from mild diarrhoea to pseudomembranous colitis [*1,2*]. *C. difficile* is a major nosocomial pathogen, responsible for up to 20% of cases of antibiotic-associated diarrhoea in industrialized countries, and is an emerging problem in developing countries such as India [*1–6*]. Infection is most commonly seen in hospitalized, elderly patients, and is almost always associated with the administration of antibiotics [*7–9*]. Searches for *C. difficile* and/or its toxins in the stools of hospitalized patients with diarrhoea are not routinely carried out in most hospitals in developing countries, including Jordan. The purpose of this study was to investigate the prevalence of *C. difficile*-associated diarrhoea among hospitalized Jordanian patients.

Methods

We studied 400 stool specimens from 400 patients admitted to Jordan University Hospital (JUH) over a 6-month period (January–July 1999). The patients comprised 222 males and 178 females. Patients under 2 years of age were excluded from the study because of the frequency of asymptomatic carriers seen in this age group.

There were 300 stool specimens obtained from inpatients with diarrhoea. These were sent to the bacteriology laboratory with a request from the treating doctor for the detection of enteric pathogens. Of these, 39 were sent with specific requests for detection of *C. difficile* or its toxins.

From the control group (100 newly admitted patients without diarrhoea, who self-reported they had not received any antibiotics prior to admission), 100 stool specimens were collected at random within 24–48 hours of admission. The age, sex and length of hospital stay from the date of admission to the date of specimen collection were recorded. Type of antibiotic treatment when present was also recorded. All stool specimens were cultured directly and enriched for the isolation of *Salmonella* spp. and *Shigella* spp. In addition, specimens were cultured for *C. difficile* and assayed for toxin A, either within 24 hours of collection, or preserved at –70 °C, and later assayed for toxin A within 2 months. All stool specimens were examined for the presence of blood, ova and parasites using a wet preparation method with 0.85% saline solution.

A 70-minute enzyme immunoassay (EIA) method using a polyclonal anti-A antibody was used for the detection of *C. difficile* toxin A in stool specimens (Culterette Brand Toxin CD kit, Becton-Dickison, Maryland, United States of America). *C. difficile* was isolated from stool specimens by inoculation directly, and after treatment, with 95% ethanol on cycloserine-cefoxitin fructose agar plates [*10*]. Inoculated plates were incubated for 48 hours at 37 °C using a Gas Pak generating system. A control reference strain of *C. difficile* (ATCC 43957) was subcultured and incubated with each run. Presumptive identification of *C. difficile* isolates was confirmed through colony appearance, catalase test, Gram stain and spore stain, and confirmed using the API 20A system (bioMérieux, Marcy l'Etoile, France) [*11*]. Stool specimens were also plated directly on Hektoen enteric agar, salmonella–shigella agar and inoculated onto selenite-F broth (Oxoid, United Kingdom) for enrichment for 24 hours, then subcultured on Hektoen enteric agar and salmonella–shigella agar [*12*].

Table 1 **Prevalence of *Clostridium difficile* and/or its toxin A in stools of clinical diarrhoeal cases and controls according to sex and age**

Status of cases	Age (years)	Males		Females		*C. difficile* or its toxin		Patients examined
	Mean ± *s*	No.	%	No.	%	No.	%	
Clinical diarrhoea	37.0 ± 19.5	164	54.7	136	45.3	29	9.7	300
Controls	35.2 ± 21.4	58	58.0	42	42.0	3	3.0[a]	100
Total		222	55.5	178	44.5	32	8.0	400

[a]*Positive* C. difficile *culture only.*
$\chi^2 = 4.529$, $P < 0.05$.
s = standard deviation.

Data were analysed using the *SPSS* to determine frequency distributions and chi-squared values. The *Z*-test was used to investigate statistical differences between two proportions. *P*-values of < 0.05 were considered significant.

Results

We examined 400 stool specimens to detect *C. difficile* or its toxin A by culture and EIA test. Of these, 29 of the 300 (9.7%) specimens obtained from hospitalized patients with diarrhoea (clinical cases), and 3 of the 100 (3.0%) control group specimens were positive for *C. difficile* or its toxin A. This difference was statistically significant ($P < 0.05$) as shown in Table 1. The prevalence of *C. difficile* and/or its toxin A among the clinical diarrhoeal cases was also significantly higher ($P < 0.05$) than the prevalence of other potential enteric pathogens of *Salmonella* spp. (2.3%), *Shigella* spp. (1%) *and Entamoeba histolytica* (2.7%), as shown in Table 2.

The specific characteristics of the 29 patients with positive *C. difficile* culture and/or its toxin A are summarized in Table 3. Of these patients, 14 (48.3%) were aged ≥ 50 years, 21 (72.4%) had been treated with antibiotics and 4 (13.8%) had been given cancer chemotherapy. Clinical diarrhoea associated with loose stools and bloody stools was found in 23 (79.3%) and 10 (34.5%) patients, respectively. Both culture and EIA toxin A test detected 22 of the 29 (75.9%) *C. difficile*-associated diarrhoea cases. An additional 5 (17.2%) of these cases showed a positive result only by EIA toxin A test. The remaining 2 cases (6.9%) were only culture-positive for *C.*

Table 2 **Prevalence of common enteric pathogens in stool specimens among 300 clinical diarrhoeal cases**

Organism	Patients with positive results	
	No.	%
Clostridium difficile[a]	29	9.7
Salmonella spp.	7	2.3
Shigella spp.	3	1.0
Entamoeba histolytica[b]	8	2.7
Total (100%)	47	15.7

[a]*All produced toxin A in culture filtrates.*
[b]*Mostly found in children.*

Table 3 **Characteristics of 29 patients with positive culture of *Costridium difficile* and/or its toxin A**

Characteristic	No.	%
Sex		
Male	17	58.6
Female	12	41.4
Age group (years)		
2–15	8	27.6
16–49	7	24.1
≥ 50	14	48.3
Clinical diarrhoea		
Soft-liquid stool	23	79.3
Normal stool	6	20.7
Bloody stool		
Present	10	34.5
Absent	19	65.5
Treatment		
Antibiotic treatment	21	72.4
Cancer chemotherapy	4	13.8
No treatment	4	13.8
Positive		
C. difficile culture only	2	6.9
Toxin A only	5	17.2[a]
Both culture and toxin A	22	75.9[a]
Mean age ± s (years)	39.2 ± 23.2	
Mean duration of hospitalization (days)	10.6 ± 8.2	
Mean duration of diarrhoea (days)	2.2 ± 1.9	
Mean duration of antibiotics (days)	6.3 ± 5.0	

[a] *27/29 (93.1%) of stool specimens of patients were positive for C. difficile toxin A using EIA test.*

difficile-producing toxin A. In addition, the mean duration of hospitalization, antibiotic treatment and duration of diarrhoea in these patients was 10.6, 6.3 and 2.2 days respectively. A significantly higher number of patients ($P < 0.05$) had developed diarrhoea due to *C. difficile* in association with third-generation cephalosporins (28.6%) and combination of antibiotics (23.8%) than any other group of antibiotics (Table 4).

Discussion

During the past decade, cases of diarrhoea caused by *C. difficile* have increased significantly among hospitalized adult patients, associated mostly with nosocomial infection, as well as intensive and prolonged use of certain antimicrobial drugs and cancer chemotherapy. The most common antimicrobial drugs implicated in *C. difficile* infection include cephalosporins, clindamycin, ampicillin, and amoxycillin [*7–9,13*]. A number of studies have reported that third-generation cephalosporins are causing more *C. difficile*-associated diarrhoea than earlier cephalosporins [*7,8,*

Table 4 **Antimicrobial treatment associated with positive *Clostridium difficile* culture and/or its toxin**

Antimicrobial drugs	No.	%
Third-generation cephalosporins[a]	6	28.6
Second-generation cephalosporins[b]	3	14.3
First-generation cephalosporins[c]	1	4.8
Antibiotic combination[d]	5	23.8
Aminopenicillins	3	14.3
Unknown druge	3	14.3
Total	21	100.0

[a] *Ceftazidime 3, cefotaxime 2, ceftriaxone 1.*
[b] *Cefuroxime 2, cefoxitin 1.*
[c] *Cephalexin 1.*
[d] *Vancomycin–amoxacillin 2, imipenem–ampicillin 1, metronidazole–lincomycin 1, ceftriazone–amphotericin 1.*

14,15]. An increased prevalence of *C. difficile*-associated diarrhoea in a hospital setting may suggest nosocomial infection. Detection of *C. difficile*-associated diarrhoea or pseudomembranous colitis among hospitalized patients is therefore important in guiding both antimicrobial treatment and control of nosocomial infection [*1,15,16*].

The prevalence of *C. difficile* and/or its toxin A among hospitalized Jordanian patients in this study was 9.7% in association with clinical diarrhoea, and 3% among the control patients without diarrhoea. This difference was statistically significant ($P = 0.033$). About 50% of our patients infected with C. *difficile* were aged ≥ 50 years. These results are generally similar to recently reported studies from some Western countries [*3,4,11,16*]. The prevalence of some enteric pathogens (in 3.3% of cases) known to be a common cause of diarrhoeal diseases in Jordan was significantly less ($P < 0.05$) than the prevalence of *C. difficile* (9.9%) in our hospitalized patients [*17*]. Most *C. difficile*-associated diarrhoea cases (93.1%) were detected using the rapid 70-minute EIA toxin A test in contrast to the 4-day culture and identification method. Of the 29 cases of *C. difficile* diarrhoea, 21 (72.4%) were associated with prior antimicrobial treatment, of which third-generation cephalosporins and combination antibiotics appeared to be the most strongly implicated. Despite this, our study points to other antimicrobials and cancer chemotherapy that might be a potential cause of *C. difficile*-associated diarrhoea under certain conditions. However, the role of cancer chemotherapy as a cause of *C. difficile* diarrhoea is not well established and must be further investigated [*2*]. Recently Ludlam et al. [*14*] have reported that restricting the use of injectable third-generation cephalosporins is a cost-effective method for reducing the incidence of *C. difficile*-associated diarrhoea.

Our study demonstrates that *C. difficile* is a common pathogen recovered from hospitalized patients in Jordan with symptomatic diarrhoeal illness or without diarrhoea as a result of the administration of antimicrobials or cancer chemotherapy. It is seen mostly in elderly patients following hospitalization of more than 5 days. Stools of such patients can be successfully screened using a rapid EIA specific for detection of *C. difficile* toxin A.

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