PSEUDO-OUTBREAK OF *Clostridium difficile* ASSOCIATED DIARRHEA (CDAD) IN A TERTIARY-CARE HOSPITAL

M. Beatriz SOUZA DIAS(1,2), Juliana YAMASHIRO(2), Vera L. BORRASCA(1), Valeska A. STEMPLIUK(3), Maria Rita E. ARAÚJO(4), Silvia F. COSTA(5) & Anna S. LEVIN(2,5)

**SUMMARY**

The objective of this study was to describe a pseudo-outbreak of *C. difficile* in a hospital, following a change in the method used to detect the toxin.

In February 2002, there were two cases of CDAD and in March 7 occurred, coinciding with a change of the test (from detection of toxin A to toxin A/B). An outbreak was suspected. Active surveillance and education of staff were started. A CDAD case was defined as a patient with acute onset of diarrhea (≥ three episodes of liquid stools) and a positive stool test. They were classified as hospital or community-acquired. Stool samples were also collected for *C. difficile* culture and isolates were typed using AP-PCR.

From March 2002 through December 2003 there were 138 cases of CDAD: 70% were hospital-acquired and among the 30% with CDAD present on admission, most (81%) came directly from the community (50% had no history of hospitalization). Fifty-two percent of hospital-acquired CDAD and 94% of cases on admission had already used antibiotics. The incidence of CDAD in hospitalized patients during surveillance was 3.3 per 1000 patient-admissions. The incidence of CDAD present on admission was 6.1/1000 patients. Sixteen isolates were typed and presented 13 different profiles.

In conclusion, the CDAD increase in our study occurred due to change in diagnostic methods and not due to an outbreak, as suspected initially. The incidence in hospitalized patients was much lower than in reported outbreaks. There were 13 molecular types suggesting that an outbreak did not occur. CDAD was largely community-acquired.

**KEYWORDS**: *Clostridium difficile*; Pseudo-outbreak; Molecular typing; Community-acquired.

**INTRODUCTION**

*Clostridium difficile* is the most frequent cause of hospital-acquired diarrhea. More recently *C. difficile* associated disease has become more severe leading to more frequent complications such as toxic megacolon and is more refractory to treatment. Since the early 2000s, a new hyper virulent strain, NAP1/BI/027, has been responsible for outbreaks in North America and Europe. Its epidemiology in Brazil is unknown and there have been no reports of outbreaks with hyper virulent strains in this country.

The objective of this study is to describe a pseudo-outbreak of *C. difficile* in a hospital, following a change in the method used to detect the toxin.

**METHODS**

Hospital Sírio Libanês is a private hospital in the city of São Paulo, Brazil. In 2002-3 it had 250 beds, divided between surgical and medical wards, two intensive care units (ICU), a semi-critical unit and an oncology unit. There was also a day-hospital, an emergency department and an outpatient clinic. Surgical and oncological patients were the majority.

Active surveillance for nosocomial infections was continuous in the ICU and oncology unit and was performed during four months, every year, in the other hospital areas (February, May, August and November). Until February 2002 the Infection Control Department did not perform active surveillance for *C. difficile* associated disease (CDAD); when CDAD was suspected by the attending physician, a stool EIA test for toxin A was requested. From March 2002 on, the hospital started to perform an EIA test for toxins A and/or B (Premier Toxin A and B, Meridian Bioscience, Cincinnati, USA).

In February 2002, there were two cases of CDAD, and in March seven cases were detected, which coincided with the new test. However, a potential outbreak was suspected and active surveillance was started in all units of the hospital, allied with education of the staff on prevention measures.

A case of CDAD was defined as a patient who presented an acute onset of diarrhea (three or more episodes of liquid stools) and a positive stool test for *C. difficile* toxin. Only the first episode was considered for each patient. CDAD cases were classified as:
- Hospital-acquired: if they occurred after 48 hours of hospitalization.
- Present on admission: if CDAD was present at hospital admission or within the first 48 hours of hospitalization, even if the patient had been hospitalized elsewhere or had received healthcare on an outpatient basis.

**Stool cultures for C. difficile**: Stool samples positive for toxin were frozen at -20 °C to be submitted later to culturing for *C. difficile*. One mL of the sample was added to 2 mL of 70% alcohol and incubated for one hour at room temperature. The mixture was inoculated in 10 mL of thioglycolate broth and maintained at 37 °C for seven days. After this period, the sample was plated on blood agar and maintained under anaerobic conditions for 48h. If there was a pure culture, it was plated on Cycloserine Cefoxitin Fructose Agar (CCFA) under anaerobic conditions. Culturing in blood agar was also performed. If growth on this selective medium occurred, one colony was inoculated in 10 mL of BHI for DNA extraction. If there was a mixed culture on the blood agar, colonies were re-inoculated in thioglycolate broth and the procedure was repeated. Antimicrobial susceptibility tests were not performed.

DNA extraction and molecular typing (genotyping) by arbitrary primed polymerase chain reaction (AP-PCR)

One colony from each culture was inoculated in 10 mL of BHI and grown for 48 h in anaerobic conditions. Then two mL were frozen at -70 °C and DNA extraction was carried out with the remaining 8 mL. Extractions of DNA were performed as described elsewhere. For typing AP-PCR was used with the arbitrary primer oligonucleotide T7 (GTAATACGACTCACTATAGG)6,11,12,24,25. The PCRs were prepared in PCR buffer (50 mM KCl, 20 mM Tris-HCl, 2.5 mM MgCl₂, 100 mg of bovine serum albumin per mL [pH 8.4]) containing 40 pmol of T-7, 1 mM of each deoxynucleoside triphosphate, 2 U Taq of DNA polymerase and one mg of DNA. The PCR profile was one step at 95 °C for five min, followed by twice at 95 °C for one min, 26 °C for one min and two min at 72 °C. After these two low stringency cycles, the arbitrary primer was heated at 95 °C for one min, 50 °C for one min, and 72 °C for two min in the subsequent 55 cycles.

Identification of *C. difficile* was confirmed using primers for gene 16S rDNA11. Products of amplification were analyzed using electrophoresis in agarose gel and staining with ethidium bromide. Genotypes were defined on the basis of DNA banding patterns. Isolates with identical patterns were considered genotypically “indistinguishable”, while those that differed by one to four bands were subtypes of the same type and differing by five or more bands were considered distinct types56.

**Outbreak control**: All healthcare workers in the hospital were re-trained in control measures, with emphasis on contact precautions and hand hygiene with chlorhexidine 2% soap and water. Alcohol based hand disinfection was discouraged in *C. difficile* patient rooms. Routine concurrent cleaning of the environment with organic chlorine (500 ppm) was initiated in case patient rooms, all intensive care units and operating rooms. Post discharge disinfection of all patient rooms was performed with the appropriate disinfectant (i.e., chlorine based). No cohorting was instituted since all the patients were in individual rooms.

**RESULTS**

From March 2002 through December 2003 there were 138 cases of CDAD. Among the 138 patients, 53% were male and the mean age was 64 years (range: 1-95); 70% of the cases were hospital-acquired, and among the 30% with CDAD present on admission, most (81%) had come directly from the community and 50% had no history of previous hospitalization. Fifty-two percent of the patients with hospital-acquired CDAD and 94% of cases present on admission had used antibiotics: 19% had used third-generation cephalosporins, 13% intravenous vancomycin, 6% a carbapenem, and 35% had used other antibiotics. Twenty-seven percent had used more than one antibiotic simultaneously. No patients received oral vancomycin prior to the diagnosis of CDAD.

The monthly distribution of CDAD cases can be seen in Figure 1. The incidence of hospital-acquired CDAD during this period was 7.9/1000 patients in ICUs; 10.5/1000 in oncology patients; and 1.3 in the other hospital units (Table 1). The incidence of CDAD present on hospital admission was 6.1/1000 patients.

**Number of Cases**

<table>
<thead>
<tr>
<th>Month/Year</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Mar</td>
<td>5</td>
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<td>Apr</td>
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<td>May</td>
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<td>Jun</td>
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<tr>
<td>Jul</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Aug</td>
<td>6</td>
<td>7</td>
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<td>Sep</td>
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<td>Oct</td>
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<td>3</td>
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<tr>
<td>Nov</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Dec</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Fig. 1 - Number of cases of *Clostridium difficile* associated disease from February 2002 through December 2003, in Hospital Sírio Libanês, São Paulo, Brazil. In March 2002, the diagnostic method used was changed from test for toxin A only, to test for toxins A and B.

Stools from all the 138 cases were cultured for *C. difficile*; however, growth only occurred in 16 samples. AP-PCR identified 13 different clonal profiles (Fig. 2). There were three pairs of isolates that were considered to present similar profiles; however, the isolates in pairs occurred 22 days, 2.3 months and 5.5 months apart. The results of molecular typing were only available in February 2004, when it became clear that this was a pseudo-outbreak.

**DISCUSSION**

The increase in CDAD observed in our study occurred due to the change in diagnostic methods and not due to an outbreak as suspected initially. There have been no reports of CDAD outbreaks in our country; however, due to reported outbreaks with the particularly virulent strain ribotype 027 (NAP1/BI/027 or CD027) in Europe and North America15,24,29, the increase in cases in a Brazilian hospital was especially worrisome and led to this investigation. In a Brazilian study performed in Rio de Janeiro, with a small number of isolates, the ribotypes identified were 014 and 1061, and the ribotype 027 has not been described in the country.

The incidence of CDAD in hospitalized patients in our study during active surveillance was 3.3 per 1000 patient admissions. This incidence is much lower than that described in other studies, including one in Dublin (21/1000 admissions) during a CD027 outbreak1, but similar to that described in a Turkish hospital (2.2 per 1000 admissions) by active surveillance in a non-outbreak setting6. In Brazil, *C. difficile* was detected...

Table 1
Distribution of cases of Clostridium difficile associated disease (CDAD) and incidence of hospital-acquired cases, from March 2002 through December 2003, in Hospital Sírio Libanés, São Paulo, Brazil

<table>
<thead>
<tr>
<th>Hospital Unit</th>
<th>Number of cases present on admission</th>
<th>Number of hospital-acquired cases</th>
<th>Total number of patients</th>
<th>Incidence of hospital-acquired CDAD (per 1000 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized patients</td>
<td>ICU</td>
<td>4</td>
<td>51</td>
<td>6,456</td>
</tr>
<tr>
<td></td>
<td>Oncology</td>
<td>7</td>
<td>19</td>
<td>1,809</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>26</td>
<td>26</td>
<td>20,000</td>
</tr>
<tr>
<td>Non hospitalized patients</td>
<td>5</td>
<td></td>
<td>0</td>
<td>826</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>96</td>
<td>29,091</td>
<td>3.3</td>
</tr>
</tbody>
</table>

ICU: intensive care unit.

The importance of detecting toxin B besides toxin A has been discussed. Initially, toxin A was reported to be responsible for the disease. However, the role of toxin B has been reviewed and is considered important, or even essential for virulence. The sudden high incidence of Clostridium difficile diarrhea observed in our study was not due to a monoclonal outbreak, as initially suspected, but to an increase in diagnosis offered by the detection of toxin A and B, thus, it was a pseudo-outbreak.

The low yield of cultures is difficult to explain. It may be due to the long time between freezing of the stool samples and culturing, false positive toxin results, or even the limited experience of our microbiology laboratory with this pathogen.

Many different typing methods have been used for C. difficile. For the early and rapid detection of outbreak situations, methods such as restriction enzyme analysis, arbitrary primed polymerase chain reaction (AP-PCR), and PCR ribotyping are commonly used. For long-term epidemiologic studies, multilocus sequence typing, multilocus variable number of tandem repeats analysis, and amplified fragment length polymorphism are of interest. AP-PCR was compared with immunoblotting and restriction endonuclease analysis, and presented similar results. Arbitrarily primed PCR, that relies on the T7 oligonucleotide, was used to identify different strains and was sensitive and accurate to type C. difficile. Our use of AP-PCR was sufficiently discriminatory, as there were 13 profiles among 16 isolates, which was enough to conclude that an outbreak did not occur.

Although originally considered a nosocomial-acquired condition, CDAD was largely community-acquired in our investigation (30%). Only half of these community-acquired cases had been hospitalized previously, but most had used antibiotics. A recent study of community-acquired CDAD in the Netherlands demonstrated a prevalence of 1.5% among stool samples sent to reference laboratories by 195 general practitioners. Most patients had not been hospitalized within the previous year, but more than half had used antibiotics within the previous six months. The cause for this presence in the community has been explained by the previous hospitalization of patients and the use of antibiotics, but this does still not explain the occurrence in our patients without these risk factors. It is possible that the presence in the community of asymptomatic carriers of C. difficile originating in hospitals has increased the community spread of this agent, especially in patients with underlying conditions.

This study has limitations. Only isolates from 16 of 138 cases (12%) were typed, but our results showed such a large variety of molecular types that is highly unlikely that a clonal outbreak was missed. However, this low positive rate was a limitation of the study. We did not compare our isolates directly with the CD027 strain, but the relatively mild clinical presentation of our cases suggests that this was not a problem in our hospital. Although the data are relatively old and therefore may not represent what occurs currently, no changes in the clinical features of C. difficile associated disease have been observed in our hospital since the occurrence of the pseudo-outbreak, which suggests that, if present, very virulent strains such as CD027, or the more recently described CD078, are not yet an important problem in our setting. However, the environmental control measures instituted during the pseudo-outbreak were kept in place until the present time.

RESUMO

Pseudo-surto de diarréia associada a Clostridium difficile (DACD) em hospital terciário

O objetivo deste estudo foi descrever um pseudo-surto de C. difficile em um hospital após a troca do método de detecção de toxina.
Em fevereiro de 2002 houve dois casos de DACD e em março ocorreram sete casos, que coincidiram com a mudança de teste (que detectava apenas toxina A e passou a detectar toxinas A e B). Foi suspeitado que houvesse um surto e vigilância ativa e reforço educacional para os profissionais de saúde foi implantado. Um caso de DACD foi definido como um paciente com início abrupto de diarreia (> 3 episódios de fezes líquidas) e um teste positivo. Os casos foram classificados como de aquisição comunitária ou hospitalar. Foram colhidas fezes para cultura para C. difficile e os isolados foram tipados por AP-PCR.

De março de 2002 a dezembro de 2003 houve 138 casos de DACD: 70% foram hospitalares e, entre os 30% de casos comunitários, a maioria (81%) foi de pacientes provenientes diretamente da comunidade (50% não tinham histórico de internação). Cinquenta e dois por cento dos casos de DCAD hospitalar e 94% de casos na admissão haviam utilizado antimicrobianos. A incidência de DCAD em pacientes internados foi de 3,3/100 pacientes e na admissão foi 6,1/1000 pacientes. Dezesseis por cento tinham histórico de internação. Cinquenta e dois por cento dos de aquisição comunitária ou hospitalar. Foram colhidas fezes para cultura para C. difficile e os isolados foram tipados por AP-PCR.

Em conclusão, o aumento de DACD no nosso estudo ocorreu por uma mudança de método diagnóstico e não devido a um surto como foi suspeitado inicialmente. A incidência em pacientes internados foi muito inferior ao que já foi relatado em surtos. Houve 13 perfis moleculares sugerindo que não ocorreu um surto. DACD foi, em grande parte, de aquisição comunitária.

REFERENCES


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