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Molecular types and antimicrobial susceptibility patterns of *Clostridium difficile* isolates in different epidemiological settings in a tertiary care center in Israel☆

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ABSTRACT

The aims of this prospective study were to examine the correlation between the molecular types and the antimicrobial susceptibility patterns of *Clostridium difficile* isolates with the source of acquisition and the occurrence of *C. difficile* infections (CDI) in a tertiary center in Israel. All available isolates from community-acquired (CA) CDI episodes (n = 43) and matching numbers of isolates from community-onset, hospital acquired (CO-HA, n = 67) and HA-CDI (n = 56) and 32 cases of recurrent CDI were typed and tested for susceptibility to vancomycin and metronidazole. The most common types were *SlpA* hr-02 (21%), *SlpA* hr-05/PCR-ribotype-014 (12%), PCR-ribotype-027 (10%) and *SlpA* cr-02 (10%). The PCR-ribotype-027 was most common in the CO-HA group and the hr-05 type was more common in the CA group. Non-susceptibility to metronidazole and/or vancomycin was found in 4/7 of re-infection isolates. Our study shows that CA-CDI is uncommon and is caused by similar strains as HA-CDI, albeit with different rates.

1. Introduction

Changes in the epidemiology and even clinical features of *Clostridium difficile* infections (CDI) are commonly attributed to particular strains, such as the PCR-ribotypes 027 and 078 strains (Gerding, 2010). The importance of specific strains was demonstrated by the parallel decline in the incidence of CDI in general and PCR-ribotype-027 CDI in particular following the implementation of prevention campaign in England and the decreased use of quinolones (Wilcox et al., 2012). The role of specific strains in relation with specific epidemiological and clinical features is less clear. For instance, although several studies have reported a high number of the PCR-ribotype-027 strain isolated in cases of relapsing CDI (Figueroa et al., 2012; Marsh et al., 2012), PCR-ribotype 027 CDI cases were not found to be more virulent compared with other types (Aitken et al., 2015). Hence, it is not clear whether the high number of relapse in PCR-ribotype 027 cases was mainly a reflection of its overall high prevalence in those populations.

In Israel the emergence of the PCR-ribotype-027 strain was associated with local outbreaks (Wiener-well et al., 2014) as well as with overall increase in CDI incidence in general hospitals and long-term care facilities (LTCF) (Adler et al., 2015). Moreover, high rates of non-susceptibility to both metronidazole and vancomycin were found in the PCR-ribotype 027 isolates, raising the concern for increased rate of treatment failures and relapse. Following these studies, our goals were to examine the correlation between the microbiologic features of *C. difficile* isolates, including the molecular type and the antimicrobial susceptibility results with two types of epidemiological and clinical classifications: a) the source of acquisition (community- vs. hospital-acquired infections) and b) with the occurrence of the CDI episode (first episode vs. recurrence) in a tertiary center in Israel.

2. Methods

2.1. Study design

The study was conducted at the Tel-Aviv Sourasky Medical Center (TASMC) during 2011–2014. In a prospective case–control study, adult patients with laboratory-confirmed (see below) *C. difficile* infection (CDI) were asked to sign an informed consent to participate in the study (by author WN) and were classified according to a) the source of acquisition and b) according to number of CDI episodes (study initiated in 2012). CDI episode was defined as at least three episode of loose stool within 24 hours and a positive laboratory diagnosis of CDI (see below). The sources of acquisition were divided into three types: 1) hospital-acquired (HA)-CDI initiated >48 hours of admission; 2) hospital-acquired, community-onset (HA-CO)-CDI initiated <48 hours of admission in patients that were admitted to a healthcare facility within the previous three months and 3) community-acquired

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(CA)- CDI initiated <48 hours of admission in patients that were not admitted to a healthcare facility within the previous three months (Centers for Disease Control and Prevention, 2008). All CA cases were included and were matched with equal (or slightly larger) number of randomly-selected patients from the two other groups. Randomization was done by including similar number of samples, from each epidemiological group, for each study year. The selection of the isolates from the isolates' freezing box was performed by a blinded observer, in order to avoid consecutive isolates. The other classification defined patients as having either a first episode of CDI (ever or within the previous 180 days) or recurrent CDI (interval of at least 14 days prior to current episode) (Marsh et al., 2012). Following molecular analysis, these cases were classified as either relapse (same type) or re-infection (different type). All cases of recurrent CDI were included and compared with first-episode CDI cases selected for the first part of the study (see above).

2.2. Microbiological methods

Laboratory diagnosis of CDI was done by testing non-formed stool samples in a two-step algorithm. The initial assay was a combined glutamate dehydrogenase antigen (GDH) and Toxin A/B immunochromatographic rapid test (C. DIFF QUIK CHEK COMPLETE, Techlab, Orlando, FL, USA); only if the results of those two tests were discordant, CDT PCR (Xpert® *C. difficile*, Cepheid, Sunnyvale, USA) was performed. Positive samples were frozen at −80 °C and thawed stool of the chosen subset of samples (see below) were inoculated on pre-reduced ChromID *C. difficile* agar plates (bioMérieux, Marcy l'Etoile, France) and incubated in an anaerobic chamber (BACTRON I-2, Shellab, OR, USA) for 48 hours. Suspicious colonies were subcultured onto sheep blood agar plates and identified based on colony morphology, typical odor and molecular tests as described below. Antimicrobial susceptibility testing (AST) was performed for vancomycin, metronidazole and moxifloxacin using the gradient method (Etest®, bioMérieux, Marcy l'Etoile, France) with the technician blinded to the isolate's type. Pre-reduced, supplemented Brucella blood agar plates (Cat. BBL255509, BD, NJ, USA) were inoculated with an inoculum of 0.5 McFarland, incubated and read after 48 hours, using the *C. difficile* ATCC 700057 as reference strain. Minimal inhibitory concentration (MIC) criteria for epidemiological cut-off (ECOF) values were interpreted according to the EUCAST recommendations: (EUCAST, 2015) Moxifloxacin ≤4 μg/ml, metronidazole ≤2 μg/ml and vancomycin ≤2 μg/ml.

2.3. Molecular methods

Identification of *C. difficile* was confirmed by detection of the species-specific gene, *tpi* by PCR (Lemee et al., 2004). The presence of the A and B toxins (*tcdA* & *tcdB*) as well as the binary toxin (*cdtB*) was tested by PCR (Persson et al., 2008). The presence of in-frame deletions in the *tcdC* gene was tested by PCR (Persson et al., 2011). The presence of the epidemic strain, BI/NAP1/027 (designated gc8 by *slpA*), was initially detected by observing the 18-bp deletion in the *tcdC* gene and the *cdtB* gene and confirmed by PCR ribotyping (Persson et al., 2011; Xiao et al., 2012). Non-027 isolates were further typed by PCR and sanger sequencing of the variable region of the *slpA* gene (Kato, 2005; Kato et al., 2010). The amino acid sequences were deduced from the DNA sequences and type was established if the deduced amino acid sequences differed from existing types by more than 20 amino acid residues. Subtypes consisted of groups differing by up to 20 amino acid residues. Designation of *slpA* types and determination of the inferred PCR-ribotype was done based on the nomenclature used by Kato (when present) (Kato, 2005; Killgore et al., 2008; Kato et al., 2010); otherwise, a new name was given. In general, the nomenclature for each type was designated by letters and of subtypes by numbers (e.g., hr-02, hr-05, etc.…). In cases were a new subtype was identified that resembled another subtype, additional letters were added (e.g., xr-03 IL-1). Isolates were also typed by ribotyping whenever the corresponding PCR-ribotype was available from reference strains, in order to provide a more familiar nomenclature. Reference strains for PCR ribotyping were kindly provided by the Cardiff-European Center for Disease Control (ECDC) collection of *C. difficile* strains.

2.4. Data collection and analysis

Data pertaining the source of acquisition was collected from the hospital's electronic data system as well as from patients by author WN. The proportion of the different strains among the groups of different acquisition source were compared by Fisher's exact test using GraphPad online software (GraphPad Software, Inc. LaJolla, CA). The study was approved by the hospital's ethical committee.

3. Results

3.1. Molecular typing and antimicrobial susceptibility results of C. difficile isolates according to acquisition source

During the study period there were 824 CDI cases that were diagnosed at the TASMC, of which 52 were CA-CDI (6.3%), 591 were HA-CDI (71.7%) and 181 were CO-HA CDI (22%). Samples were available in 43 CA-CDI cases; accordingly, 67 and 56 cases of HA-CDI and CO-HA-CDI isolates were selected for comparison. The *SlpA* types, PCR-ribotypes and antimicrobial susceptibility results of these 166 isolates are presented in Table 1; the distribution of the different *SlpA* types according to acquisition source is presented in Fig. 1. All *C. difficile* isolates were tested positive by *tcdA* & *tcdB* PCR's. The Binary toxin gene was detected in all PCR-ribotype 027 and −078 strains. The four most common types comprising together 50% of the isolates were *SlpA* hr-02 (21%), *SlpA* hr-05/PCR-ribotype-014 (12%), PCR-ribotype 027 (10%) and cr-02 (10%). The PCR-ribotype 078 strain was identified in only 5 isolates. The distribution of two of the common types differed significantly between the acquisition groups: a) the 027 PCR-ribotype was most common in the CO-HA group compared with the two other acquisition groups (23% vs. 4.5%, $p < 0.001$) and b) the hr-05 type was more common in the CA group compared with the two other acquisition groups (23% vs. 8.8%, $p = 0.013$). There was no significant change in the molecular types over the study period.

Non-susceptibility (NS) to antimicrobials was associated with particular types and hence was not correlated with the acquisition sources: NS to metronidazole was found only in the PCR-ribotype-027 isolates (39%), NS to vancomycin was found in the PCR-ribotype-027 isolates (61%) as well as the cr-02 type (82%) and resistance to moxifloxacin was universal in these two types as well as in the *SlpA* fr-02/PCR-ribotype 017 and very common in the PCR-ribotype 078 isolates (80%).

3.2. Molecular typing and antimicrobial susceptibility results of C. difficile isolates from recurrent infection

During the study period there were 82 cases (10%) of recurrent CDI that were diagnosed at the TASMC, of which more than one sample was available for analysis in 32 cases. The majority of these cases (n = 25, 78%) were identified as relapse (Table 2) and the remainder (n = 7, 22%) were re-infection (Table 3).

Relapse cases in all but one case were hospital-acquired, mostly due to the same types that were common overall, with the exception of the *SlpA* hr-05 type that was identified in only one case. Resistance to metronidazole and vancomycin was more common in these cases: in addition to the PCR-ribotype 027, NS to metronidazole was found in 25% of the *SlpA*-02 isolates and in 66.7% of the fr-01 isolates. With the exception of one case, the MIC to metronidazole and vancomycin remained identical or within two-fold dilution in the second (recurrent) isolates.

Most episodes of re-infection were HA and one was CO-HA. As with relapse, the representation of strains was similar to the overall