Prevalence and diversity of *Clostridium difficile* strains in infants

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During early infancy asymptomatic intestinal colonization by *Clostridium difficile* is frequent. To update information on infant colonization prevalence and to characterize infant strains, in terms of their virulence factors and their phylogenetic diversity, a prospective screening of *C. difficile* in the stools of infants 0 to 2 years old was conducted at Jean Verdier Hospital (Hôpital Jean Verdier) over an 18 month period. *C. difficile* was screened by toxigenic culture, and molecular characterization was performed by PCR-ribotyping and multilocus sequence typing (MLST). The overall *C. difficile* colonization prevalence was 33.7 % (99/294). The colonization rate by a toxigenic strain was 7.1 % (21/294). Community-acquired *C. difficile* accounted for 66.7 % (66/99) of cases. Molecular typing was performed on 90 isolates from Jean Verdier Hospital and 8 additional isolates from another hospital in Versailles (Centre Hospitalier de Versailles). Among these isolates, 23 were toxigenic (2 *tcdA*/tcdB and 2 *tcdA*/tcdB). All the isolates were negative for the binary toxin genes. Seventeen PCR ribotypes (PRs) were identified, with five PRs accounting for 82.7 % (81/98) of the isolates. MLST generated 15 different sequence types (STs). The predominant genotype, PR027-ST3 (33.7 %), included only non-toxigenic strains. Toxigenic strains were distributed in eight genotypes. Neither PR027-ST3, nor PR078/126-ST49 were identified but some PRs/STs corresponded to well-known adult infectious strains. These results indicate that infants are widely colonized by non-toxigenic strains. However, toxigenic adult infectious strains circulate in asymptomatic infants even in the community; thus, infants may be a reservoir for adult infectious strains.

**INTRODUCTION**

*Clostridium difficile* can colonize the gut due to its colonization factors (Denève et al., 2009). Then, *C. difficile* can produce its main virulence factors, the two large clostridial toxins TcDA and TcDB. This leads to asymptomatic carriage or a wide spectrum of diseases, ranging from mild diarrhoea to severe pseudomembranous colitis (Rupnik et al., 2009). Asymptomatic carriage occurs in 3 to 5 % or more of adults (McFarland et al., 2000). This percentage is higher, reaching 20 %, in hospitalized and antibiotic-treated patients (McFarland et al., 1989). In infants under 2 years of age, asymptomatic colonization is frequent, with a prevalence of 60 % or more depending on infant age and the environment (Bryant & McDonald, 2009; Collignon et al., 1993). After 2 years of age, the prevalence becomes similar to that of adults (McFarland et al., 2000).

Infection is uncommon in young children, and several hypotheses have been advanced to explain this asymptomatic state. The first one is the competitive intestinal colonization by non-toxigenic strains, another one is the immaturity of the immune system and of the intestinal tract associated with possible absence of toxin receptors, and others include modulation of toxin production by the infant microbiota and toxin neutralization by maternal antibodies (McFarland et al., 2000). However, *C. difficile* has also been reported to cause a wide spectrum of diseases in children from birth to adolescence, such as acute or
chronic diarrhoea and rarely severe colitis (McFarland et al., 2000). The role of \textit{C. difficile} in childhood diarrhoea appears to be closely related to age (Zilberberg et al., 2010). Symptomatic cases are clearly described in pre-term infants, newborns and children older than 3 years old. In contrast, the pathogenic role of \textit{C. difficile} in infants under 2 years is highly controversial and needs further examination (Klein et al., 2006).

Recently, the epidemiology of \textit{C. difficile} infection (CDI) has globally changed, with an increase in the incidence and emergence of hypervirulent strains, such as the PCR ribotype (PR) 027 strain (Belmares et al., 2009; Coignard et al., 2006). In children, reported cases of CDI have also increased (Kim et al., 2008; Zilberberg et al., 2010). Consequently, the epidemiology of \textit{C. difficile} may also be changing in infants.

In this context, we studied \textit{C. difficile} intestinal colonization in young children (<2 years old) and characterized the isolates. The diversity of the strains was analysed by PCR-ribotyping and multilocus sequence typing (MLST) in order to determine the phylogenetic relationships between the infant and adult strains, and to better understand the role of the intestinal colonization by \textit{C. difficile} in infants.

**METHODS**

**Subjects and samples.** A screening for \textit{C. difficile} was performed at Jean Verdier Hospital (Hôpital Jean Verdier), in the Paris suburbs, on all stool samples sent to the laboratory for stool analysis from infants aged 0 to 2 years over an 18 month period (July 2008–December 2009). The infants were outpatients consulting in the paediatric unit and the paediatric emergency unit, and inpatients hospitalized in the paediatric ward at Jean Verdier Hospital. Stool samples were collected from the diapers or after defecation. One specimen was studied per infant. The Evaluation Committee of Ethics of Biomedical Research Projects (CEERB) of the Northern University Hospital Group of Paris approved the protocol for the present study (no. 09-005).

**Clinical data collection.** Clinical data were collected from medical charts and the hospital database. For all infants, sex, date of birth and the date of each analysis were noted. To determine the most likely mode of \textit{C. difficile} acquisition, additional information was collected for \textit{C. difficile} carriers: history of hospitalization in the past 3 months or since birth for infants under 3 months of age and the duration of hospitalization prior to the sampling. Community-acquired \textit{C. difficile} corresponded to the absence of hospitalization in the previous 3 months coupled with a hospital stay not exceeding 48 h at the time of sampling. Hospital-acquired \textit{C. difficile} corresponds to a history of hospitalization and/or duration of hospitalization exceeding 48 h at the time of sampling. For carriers of a toxigenic \textit{C. difficile} strain, diarrhoea (defined as at least three loose stools per day) was noted as well as the suspected aetiology.

**Detection and identification of \textit{C. difficile}.** Faecal samples were tested for the presence of \textit{C. difficile} using toxigenic culture, considered as a reference method, on fresh stools. The selective culture and \textit{C. difficile} identification were performed as previously described (Rousseau et al., 2010). Isolated strains were conserved in 15% (v/v) glycerol at −80 °C. Eight additional \textit{C. difficile} strains were obtained from the stools of infants hospitalized at Centre Hospitalier de Versailles in another suburb of Paris.

**DNA extraction.** Bacteria obtained after 48 h anaerobic blood agar culture were suspended in 1 ml distilled water in a microcentrifuge tube and boiled for 20 min. After the removal of cellular debris by centrifugation (15 000 g for 2 min), the supernatant containing the genomic DNA was used for PCR amplification.

**Detection of tcdA, tcdB and binary toxin genes.** Toxin-encoding genes were screened by PCR following the procedure described by Lemée et al. (2004b) for \textit{tcdA} and \textit{tcdB}, and by Stubbs et al. (2000) for \textit{cdtA} and \textit{cdtB} (binary toxin). PCR amplifications were carried out in a final volume of 50 μl. PCR products were analysed by electrophoresis on a 1.5% agarose gel (SeaKem) containing ethidium bromide (0.5 μg ml⁻¹) (Eurobio).

**PCR ribotyping.** Specific oligonucleotide primers complementary to the 3’ end of the 16S rRNA gene (5’-GTGCGGCTGGATCACCCTGCT-3’) and the 5’ end of the 23S rRNA gene (5’-CCCTGGACCCCTTAATACCTGACC-3’) were used to amplify the variable-length intergenic spacer region. Amplification by PCR was performed as described by Bidet et al. (2000). PCR products were separated according to their specific sequence by electrophoresis in a 3% Resolphor agarose gel (Eurobio) containing ethidium bromide (0.5 μg ml⁻¹) for 4 h at 100 V.

PR profiles were analysed with GelCompar software (version 2.2; Applied Maths). A 100–1000 bp DNA ladder (BioLabs) was used to normalize the profiles. This marker allows correction of any distortion in the gel migration and comparison of profiles between gels.

PR profiles were compared with those of the following reference PRs according to Brazier’s nomenclature: 027, 001, 078, 106, 163, 017, 077/020/014, 048/012, 015, 010 and 094. When a PR profile was found to be identical to the PR profile of an available reference strain, the Brazier’s nomenclature was used. In other cases, a site-specific nomenclature of the Jean Verdier Hospital, with the JV prefix followed by a number, was adopted.

**MLST.** MLST was performed according to the procedure described by Lemée et al. (2004a), slightly modified according to the new MLST scheme available at www.pasteur.fr/recherche/genopole/PPH8/mlst/. The seven housekeeping genes targeted are now: \textit{aroE}, \textit{dutA}, \textit{gmk}, \textit{groEL} (instead of \textit{ddl} in the previous scheme), \textit{recA}, \textit{sodA} and \textit{tpi}. Primers, PCR and sequencing protocols are available on the website, which also provides many tools for MLST data processing. The dendrogram based on concatenated sequences homology was built using the neighbour-joining method and bootstrapping algorithms contained in the MEGA software version 2.1 (Kumar et al., 2001).

**Antimicrobial susceptibility testing.** The susceptibility of \textit{C. difficile} strains to the following antibiotics was tested: metronidazole, vancomycin, erythromycin, moxifloxacin, linezolid and tetracycline. The agar disc diffusion method was used and susceptibility to metronidazole and vancomycin was checked by Etest. Interpretation was done according to the recommendations of the Comité de l’Antibiogramme de la Société Française de Microbiologie. The breakpoint diameters (Ds) for categorizing strains as susceptible were as follows: Ds ≥21 mm for metronidazole, Ds ≥17 mm for vancomycin, Ds ≥22 mm for erythromycin, Ds ≥21 mm for moxifloxacin, Ds ≥28 mm for linezolid and Ds ≥19 mm for tetracycline.

**RESULTS**

**Prevalence of intestinal colonization**

A prospective screening of intestinal \textit{C. difficile} colonization was conducted among 0 to 2 years old infants at the
Hospital Jean Verdier. A total of 294 infants (sex male/female=1.07) were tested for faecal C. difficile presence. C. difficile was isolated in the stools of 99 infants (33.7%). Twenty-one of the C. difficile carriers were colonized with a toxigenic strain. Colonization prevalence by a toxigenic strain was therefore 7.1% (21/294). The distribution of infants according to age was as follows: 62 newborns aged 0–1 month, 189 infants aged 1 month–1 year, and 43 infants aged 1–2 years. Colonization prevalence was 25.8% (16/62, with 3 toxigenic strains) for newborns, 37.6% (71/189, with 13 toxigenic strains) for infants aged 1 month–1 year, and 27.9% (12/43, with 5 toxigenic strains) for infants aged 1–2 years old. The colonization rate reached a maximum of 72.2% (13/18) for infants aged between 7 and 9 months. The proportion of toxigenic strains significantly increased from newborns (3/16, 18.8%) and infants aged 1 month–1 year (13/71, 18.3%) to infants aged 1–2 years old (5/12, 41.6%). Infants were equally colonized regardless of sex (female/male=49/50). Toxigenic strains were also indifferently distributed between girls and boys (female/male=10/11).

No case of CDI was diagnosed among carriers of a toxigenic strain. Most of them (17/21) showed no diarrhoea, 2 had a rotavirus infection and 2 had self-limiting diarrhoea without common conventional viral (rotavirus or adenovirus) or bacterial aetiology.

Among the 99 colonized infants, 76 (76.8%) had no history of hospitalization within the previous 3 months or since birth, and 71 (71.7%) were outpatients or were sampled during the first 48 h of hospitalization. Therefore, community-acquired C. difficile accounted for 66.7% (66/99) of cases. The other 33 carriers, with at least one risk factor for nosocomial-acquisition, were categorized as hospital-acquired C. difficile. Community-acquired C. difficile was also the main mode of acquisition whatever the strain, toxigenic (14/21, 66.7%) or not (52/78, 66.7%).

Molecular typing
A total of 98 C. difficile strains isolated from infants aged 0–2 years were analysed at the molecular level. Indeed, 90 strains were isolated at Jean Verdier Hospital (9 strains, including a toxigenic strain, could not be typed for technical reasons). In addition, eight strains were isolated from the stools of 0–2 years old infants from a hospital in Versailles (Centre Hospitalier de Versailles), in order to include isolates from a different geographical area. Three of the eight strains were toxigenic. Therefore, 98 isolates were typed of which 23 were toxigenic. Detection of toxin-encoding genes revealed that 21 isolates, including the 3 isolates from the Versailles hospital, were tcdA+/tcdB+ and 2 were tcdA−/tcdB+. No strain harboured the binary toxin-encoding genes. Strain characteristics are summarized in Table 1.

Seventeen PRs were identified, with five PRs accounting for 82.7% (81/98) of the isolates. Among the isolates, MLST generated 15 different sequence types (STs). An excellent agreement was found between the two methods. Strains belonging to a given PR corresponded always to the same ST and vice versa except in two cases. Indeed, ST38 strains belonged to PRJV11 and PRJV28, and ST10 strains belonged to PRJV17 and PRJV33. Furthermore, in the same PR/ST genotype the toxigenic status was the same for all the strains. The most represented genotypes were in order of frequency: PRJV11-ST38 (33.7%), PR014/020/077-ST33 (15.3%), PR010-ST13 (13.3%), PRJV28-ST38 (11.2%) and PRJV20-ST37 (9.2%). These genotypes included only non-toxigenic strains, except the PR014/020/077-ST33 genotype that contains toxigenic tcdA+/tcdB+ isolates. Toxigenic strains were distributed into eight PR/ST genotypes. Moreover, all the genotypes were represented by at least five strains, including community-acquired and hospital-acquired isolates.

Relationships between infant and adult strains
Genotypes of infant strains were compared with those of adult strains belonging to three strain collections, mainly recovered during CDI. The first adult strain collection was from Jean Verdier Hospital and corresponded to 126 C. difficile strains belonging to 27 PRs isolated from adult patients between 2001 and 2009. The second collection was from the Rouen hospital (Centre Hospitalier Universitaire de Rouen), located in another region of France, and corresponded to 362 C. difficile strains isolated from adult patients. The third one corresponds to the web MLST database of C. difficile, hosted at www.pasteur.fr/recherche/genopole/PF8/mlst/, which includes strains isolated from several continents and countries (539 strains belonging to 57 STs, at the end of December 2010, from 17 different European countries and the USA) (Fig. 1).

For the same period and location, at Jean Verdier Hospital, infant genotypes were compared to adult genotypes by PCR-ribotyping. Considering PRs represented by at least 2 isolates, all infant PRs were recovered among adult strains except the non-toxigenic PRJV28 (11 isolates) exclusively found among infant strains. Thus, at the local level the same strains circulated in adults and infants. STs of infant strains from the study were compared with STs of adult strains from the international MLST database and the Rouen hospital (Centre Hospitalier Universitaire de Rouen) collection. For all infant genotypes, adult strains were recorded, except for the PRJV32-ST65 genotype. Neither toxigenic PR027-ST3, nor toxigenic PR078/126-ST49 were identified among strains colonizing infants. However, toxigenic infant strains disseminated in many lineages some of which corresponded to well-known adult infectious strains as PR014/020/077-ST33, PR016-ST26, PR012/048-ST27, PR015-ST39 and PR017-ST45.

Antimicrobial susceptibility
All strains were susceptible to metronidazole, vancomycin and linezolid (Table 1). Considering genotypes represented
by more than two isolates, erythromycin resistance was identified in PRJV11-ST38 (58%), PRJV10-ST13 (46%), PRJV28-ST38 (10%) and PR014/020/077-ST33 (7%). Although clustering into the ST38, isolates belonging to PRJV11 and PRJV28 differed by their rate of resistance to erythromycin. A low rate of moxifloxacin resistance was observed with a maximum of 10% resistance in PRJV28-ST38, 7% in PR014/020/077-ST33 and 3% in PRJV11-ST38. Almost all strains were tetracycline susceptible except 6% of strains belonging to PRJV11-ST38.

**DISCUSSION**

In this study, the targeted population was children under 2 years of age. At this age, *C. difficile* is frequently present in the gastrointestinal tract but rarely associated with disease. However, while *C. difficile* has long been considered a major agent of nosocomial infections, recently community cases have emerged in adult and paediatric populations (Baker *et al.*, 2010; Benson *et al.*, 2007; CDC, 2008; Pituch, 2009; Rexach *et al.*, 2006). The study was conducted in three steps. First, the prevalence of *C. difficile* carriage in infants by toxigenic culture was determined. In the second step, the genetic diversity of *C. difficile* strains was analysed by two molecular typing methods. Then, to highlight their particularities, infant strains were compared to adult strains.

A 33.7% prevalence of *C. difficile* colonization was reported among infants 0–2 years old. This result is consistent with those of previous studies in European and North-American countries, where asymptomatic carriage rates in infants under 2 years of age were found to be high, varying from 24 to 75% (Bacon *et al.*, 1988; Brazier, 1998; Collignon *et al.*, 1993; Matsuki *et al.*, 2005; Rexach *et al.*, 2006; Tullus *et al.*, 1989; Yamamoto-Osaki *et al.*, 1994). The great variability observed in carriage rates corresponds to the 0–1 year period, while at the age of 2 years, colonization is about 37–46% regardless of the study (Collignon *et al.*, 1993; Matsuki *et al.*, 2005; Torres *et al.*, 1989; Yamamoto-Osaki *et al.*, 1994). In this study, the carriage rate of *C. difficile* was 35% for infants under 1 year of age, and 28% for infants between 1 and 2 years. However, at the age of 7–9 months, the colonization rate reached a very high level (72%) as previously described among infants under 1 year of age.

In this study, the intestinal colonization was community acquired in at least 67% cases (toxigenic or non-toxigenic strains). It is likely that this proportion was underestimated as all cases with at least one risk factor for nosocomial acquisition were classified as hospital-acquired. A recent study reported

### Table 1. Characteristics of the 98 *C. difficile* strains isolated from infants’ stools in this study: genotype, toxin profile, antimicrobial susceptibility and acquisition mode

<table>
<thead>
<tr>
<th>No. of isolates (%) [total no. = 98]</th>
<th>PR*</th>
<th>ST</th>
<th>tcdA/tcdB</th>
<th>Binary toxin (cdtA/cdtB)</th>
<th>E (% R)</th>
<th>MXF (% R)</th>
<th>TE (% R)</th>
<th>MTR (% R)</th>
<th>VA (% R)</th>
<th>LZD (% R)</th>
<th>Acquisition (CA/HA)</th>
</tr>
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<tbody>
<tr>
<td>33 (33.7)†</td>
<td>JV11</td>
<td>38</td>
<td>−/−</td>
<td>−/−</td>
<td>58</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23/10</td>
</tr>
<tr>
<td>15 (15.3)†</td>
<td>014/020/077</td>
<td>33</td>
<td>+/+</td>
<td>+/+</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9/6</td>
</tr>
<tr>
<td>13 (13.3)</td>
<td>010</td>
<td>13</td>
<td>−/−</td>
<td>−/−</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11/2</td>
</tr>
<tr>
<td>11 (11.2)</td>
<td>JV28</td>
<td>38</td>
<td>−/−</td>
<td>−/−</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9/2</td>
</tr>
<tr>
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<td>JV20</td>
<td>37</td>
<td>−/−</td>
<td>−/−</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3/6</td>
</tr>
<tr>
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<td>10</td>
<td>−/−</td>
<td>−/−</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>4/0</td>
</tr>
<tr>
<td>2 (2.0)</td>
<td>017</td>
<td>45</td>
<td>−/+</td>
<td>−/+</td>
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</tr>
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<td>1/0</td>
</tr>
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<td>+/+</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>1/0</td>
</tr>
<tr>
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<td>+/+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/0</td>
</tr>
<tr>
<td>1 (1.0)</td>
<td>JV32</td>
<td>65</td>
<td>−/−</td>
<td>−/−</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>1/0</td>
</tr>
<tr>
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<td>−/−</td>
<td>−/−</td>
<td>100</td>
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<td>58</td>
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CA, Community-acquired *C. difficile*; E, erythromycin; HA, hospital-acquired *C. difficile*; LZD, linezolid; MTR, metronidazole; MXF, moxifloxacin; R, resistance; TE, tetracycline; VA, vancomycin.

*†Brazier’s nomenclature was used for PR designation when the PR profile was found to be identical to that of an available reference strain (027, 001, 163, 078, 106, 017, 077/020/014, 048/012, 015, 010, 094 as for Brazier’s nomenclature), otherwise an internal Jean Verdier Hospital nomenclature, a JV prefix followed by a specific number, was adopted.

‡Presence of strains from Versailles Hospital: four strains for PRJV11-ST38, three strains for PR014/020/077-ST33, one strain for PRJV17-ST10.
Fig. 1. Phylogenetic relationships between the STs of C. difficile strains recovered from infants in this study and all the STs registered in the web MLST database and in the Rouen Hospital collection as of the end of December 2010. STs found among strains from infants are indicated in bold. Additional data about the most frequent C. difficile toxigenic isolates recovered from adults in a nationwide survey in France in 2009 are given on the left on the dendrogram (B. Coignard and others, unpublished data). The PRs were designated either according to Brazier’s nomenclature when reference strains were available in our lab, or according to a site-specific nomenclature of Jean Verdier Hospital with a JV prefix followed by a ribotype number. JVH, Jean Verdier Hospital collection of C. difficile strains; RH, Rouen Hospital collection of C. difficile strains; MLST db, MLST database of C. difficile strains isolated in various European countries and the USA (hosted at www.pasteur.fr/recherche/genopole/PF8/mlst/); NF, not found.
rates to be as high among outpatients as among inpatients in paediatric units (Pinto et al., 2003). Thus, the diffusion of C. difficile in the community appears to be high.

In this study, 7.1% of the infants were colonized with a toxigenic strain. The majority of the toxigenic strains were tcdA+/tcdB+ and only two were tcdA−/tcdB+. All the other strains were tcdA−/tcdB−. No strain possessed the binary toxin genes. This result confirms the absence of PR027 and PR078/126 strains among the isolates. An extensive analysis of clinical data to determine the putative involvement of C. difficile in intestinal infection was conducted and no CDI was diagnosed. Klein et al. (2006) found toxigenic C. difficile in 6.7% of cases among infants with diarrhoea; and in two case–control studies, carriage rates of toxigenic C. difficile strains were shown to be similar in symptomatic and in control infants (4.2–4.5%) (Cerquetti et al., 1995; Torres et al., 1984). As it seems as common to isolate C. difficile in symptomatic diarrhoea as in asymptomatic infants, this raises the question of the involvement of C. difficile in clinical signs of diarrhea.

The reasons that explain why the colonization rates are so high and why there is a lack of pathogenicity of toxigenic strains in early infancy remain enigmatic. Miyazaki et al. (1992) showed that C. difficile existed mainly as spores and non-vegetative forms in infants’ faces. The infant microbiota could provide an environment unfavourable to spore germination. However, the microbiota might be more or less permissive to the implantation of C. difficile. We recently demonstrated that the presence of some bacteria, such as bifidobacteria, was correlated with the absence of C. difficile in the intestinal ecosystem of infants (Rousseau et al., 2011).

Few studies have characterized at a molecular level C. difficile infant strains, which are non-toxigenic most of the time. Here, we studied the diversity of infant strains and their phylogenetic relationships to adult strains by two molecular typing methods, PCR-ribotyping and MLST. MLST is highly transportable from laboratory to laboratory and allows comparison of isolates from diverse origins. A total of 17 different genotypes (PR/ST combinations) were identified among the 98 isolates. A perfect agreement was found between PCR-ribotyping and MLST. Several PRs could not be named according to Brazier’s nomenclature. In contrast, all the MLST profiles but one (a new ST identified in this work) were represented in the MLST database (MLST database of Clostridium difficile strains isolated in various European countries and the USA hosted at www.pasteur.fr/recherche/genopole/PF8/mlst/).

At the local level, at Jean Verdier Hospital, infant strains were found to be similar to adult strains, except for the non-toxigenic PRJV28-ST38 genotype only identified among infant strains. Furthermore, the non-toxigenic PRJV11-ST38 genotype was greatly represented among strains isolated in infants. These results suggest that some non-toxigenic clones may be particularly adapted to infants. An over-representation of some PRs that would be more prevalent in the geographical area covered in the study cannot completely be excluded. However, the ST38, which was present both at Jean Verdier Hospital and at Versailles Hospital, was largely distributed in the Rouen area and in the MLST database. These results concerning non-toxigenic strains are difficult to compare to others because studies on non-toxigenic strains are rare. Concerning the toxigenic genotypes found among infant strains, some, such as PR014/020/077 and PR012/048, correspond to the most frequently isolated strains from symptomatic adults with CDI. These strains, pathogenic in adults, have been isolated in an asymptomatic carriage state.

On a larger scale, all the infant genotypes described in this study, including the PRJV28-ST38, are represented in the MLST database and correspond to adult strains isolated at the Rouen hospital, located in a different region of France. The phylogeny of infant strains shows that these strains are scattered into several lineages, and randomly distributed among adult strains without any phylogenetic originality.

In conclusion, the same strains circulate in adults and in infants, including toxigenic isolates. Infants may therefore constitute a reservoir of infectious strains for adults, and act as an efficient vector of C. difficile spores. The high colonization rate in infants might be explained by contamination by C. difficile spores in the environment either in the hospital or in the community, combined with an ineffective barrier effect of the immature infant intestinal microbiota. The lack of pathogenicity in infants does not appear to be related to specific strains that circulate in infants, but rather to host factors such as the composition of the resident microbiota or the absence of toxin receptors. Further studies are, however, necessary to understand the role of C. difficile in infants and to distinguish colonization from infection.

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