J Infect Chemother 20 (2014) 307–311

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

Characteristics of *Clostridium difficile* colonization in Japanese children

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ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form
17 January 2014
Accepted 17 January 2014

Keywords:
Clostridium difficile
Children
Colonization
Tube feeding

ABSTRACT

In children, asymptomatic colonization with *Clostridium difficile* is well known, but its prevalence in Japanese children is not fully understood. The objective of this study was to determine the colonization rate of *C. difficile* and to identify the risk factors for *C. difficile* colonization in Japanese children. Single fecal samples were prospectively collected from children hospitalized in Saitama City Hospital between August 1, 2012, and March 31, 2013. Samples were obtained from neonates, at 4–14 days after birth, and from non-neonatal children, principally within 2 days after admission, to determine community-associated colonization. The fecal samples were cultured for *C. difficile*, and isolated strains were tested for production of Clostridial toxins A/B. In 95 neonates, the colonization rate of *C. difficile* was 0%. The 251 non-neonatal children were divided into two subgroups, depending on the presence or absence of underlying disease. In the subgroup without underlying disease, the colonization rates of *C. difficile* and toxin-positive *C. difficile* were 21.6% and 9.0%, respectively, while in the subgroup with underlying disease, values were 30.8% and 23.1%, respectively. The proportion of toxin-positive *C. difficile* in all of the culture-isolated strains from the latter subgroup (75.0%) was statistically higher than that from the former subgroup (41.9%) ($P = 0.049$). Multivariate logistic regression analysis indicated an association of tube feeding with significantly higher colonization rates of *C. difficile* (Odds Ratio(OR) = 24.28; 95% confidence interval(CI)[4.70–125.34]; $P < 0.001$) and toxin-positive *C. difficile* (OR = 8.29; 95%CI[1.87–36.84]; $P = 0.005$). Further evaluations are recommended to assess the epidemiology and the role of *C. difficile* in Japanese children.

1. Introduction

In 1935, Hall and O'Toole first isolated a gram-positive, cytotoxin-producing anaerobic bacterium from the stool of healthy neonates and described it as *Bacillus difficilis* because the species was difficult to isolate and culture [1]. The organism is now called *Clostridium difficile* and is known as the most important pathogen of antibiotic-associated diarrhea and colitis in adults [2]. While asymptomatic colonization by *C. difficile* is common and does not necessarily develop into *C. difficile* infection in neonates and children [3–6], the actual epidemiology of *C. difficile* colonization in Japanese children remains poorly characterized.

The objective of this study was to determine the Japanese pediatric colonization rate of *C. difficile*, and to identify the risk factors for *C. difficile* colonization in Japanese children.

2. Patients and methods

2.1. Sample collection

Single fecal samples were prospectively collected from neonates who were born in Saitama City Hospital between October 1, 2012, and December 31, 2012, and from children aged 15 years or younger who were admitted to the pediatric ward and the pediatric surgery ward at Saitama City Hospital between August 1, 2012, and March 31, 2013. We collected samples from neonates at 4–14 days after birth, a time point just before healthy neonates were discharged from the hospital. As for non-neonatal children, we principally collected samples within 2 days after admission to evaluate the community-associated colonization, while sixty-one of 251

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1341-321X/$ – see front matter
http://dx.doi.org/10.1016/j.jiac.2014.01.009

samples (24.3%) were collected at 3–7 days after admission because these children had no stools within 2 days.

2.2. C. difficile culture

Following collection, fecal samples were mixed with an equal volume of 99% ethanol and incubated for 60 min at room temperature. Then, 100 μl of each sample was cultured anaerobically on cycloserine-cefoxitin-mannitol selective agar (CCMA-EX®) (Nissui Pharmaceutical, Tokyo, Japan) for four days at 37 °C. Isolated colonies were suspended in dilution buffer to a turbidity equivalent to the McFarland standard no. 4 and tested for toxin A/B using the C.DIFF QUIK CHEK COMPLETE® (Alere Medical, Tokyo, Japan).

2.3. Colonization rate of C. difficile

We classified children into 6 age groups: neonates, 1–5 months, 6–11 months, 12–23 months, 2–5 years, and 6–15 years. We then calculated the colonization rate of *C. difficile* and toxin-positive *C. difficile* by age group. Non-neonatal children were divided into two subgroups, depending on the presence or absence of underlying disease, and the colonization rate in each subgroup was calculated.

2.4. Risk factors for C. difficile colonization

We examined the risk factors for *C. difficile* colonization among non-neonatal children. We evaluated the possible association of colonization with sex, age, antibiotic exposure, underlying disease, tube feeding, surgical history, and family living together (live-in grandparents and/or brother/sister). We defined antibiotic exposure as the use of oral or parenteral antibiotics just before admission and/or during the interval from admission to the time of sample collection. We defined underlying disease as any disease that required children to visit hospital repeatedly.

2.5. Statistical analysis

Data were analyzed using SPSS Statistics® 21.0 software (SPSS, Chicago, IL, USA). We compared the proportion of toxin-positive *C. difficile* in all of the culture-isolated strains between two subgroups by using chi-square test. Univariate analysis was performed to screen the risk factors for *C. difficile* colonization by using the chi-square test. Multivariate logistic regression analysis was performed to determine the factors associated with *C. difficile* colonization, using covariates that were hypothesized potentially to be associated with colonization in previous reports. *P* values less than 0.05 were considered statistically significant.

3. Results

A total of 346 fecal samples were tested. The baseline demographic and clinical characteristics of the children are shown in Table 1. Ninety-five fecal samples were from neonates (48 boys and 47 girls), and 251 samples were from non-neonatal children (144 boys and 107 girls). Of the 95 neonates, 26 (27.4%) were only breast-fed, 67 (70.5%) were both breast- and formula-fed, and two (2.1%) were only formula-fed. In non-neonatal children, the rates of subjects with antibiotic exposures, underlying disease, tube feeding, and surgical history, were 24.7%, 20.7%, 8.4%, and 13.5%, respectively. The rates of children living with their brother/sister and grandparents were 68.5% and 10.4%, respectively.

In neonates, the colonization rate of *C. difficile* was 0% (0/95). As for non-neonatal children, *C. difficile* colonization rates by age group are shown in Fig. 1. When all the subjects were included, the colonization rates of *C. difficile* and toxin-positive *C. difficile* were 23.5% and 12.0%, respectively (Fig. 1a). Next, we divided the subjects into two subgroups, depending on the presence or absence of underlying disease. In the subgroup without underlying disease, the colonization rate of *C. difficile* was 21.6%, with a peak of 48.5% at 12–23 months, whereas that of toxin-positive *C. difficile* was 9.0%, with a peak of 21.0% at 12–23 months (Fig. 1b). On the other hand, in the subgroup with underlying disease, the colonization rates of *C. difficile* and toxin-positive *C. difficile* were 30.8% and 23.1%, respectively. In this subgroup, children in the age groups of 2–5 years and 6–15 years, as well as 12–23 months, showed high frequencies of *C. difficile* colonization (30–38.5%), unlike the subgroup without underlying disease. The proportion of toxin-positive *C. difficile* in all of the culture-isolated strains from the subgroup with underlying disease (75.0%) was statistically higher than that from the subgroup without underlying disease (41.9%) (Odds Ratio (OR) = 4.17; 95% confidence interval (CI) [1.15–15.04]; $P = 0.049$).

Table 1
Baseline demographic and clinical characteristics of the children.

	Variable	No.	%
Age	<1 M	95	27.5%
	1–5 M	29	8.4%
	6–11 M	19	5.5%
	12–23 M	43	12.4%
	2–5 Y	102	29.5%
	5–15 Y	58	16.8%
Sex	Male	192	55.5%
	Female	154	44.5%
Nutrition[a]	Breast-fed	26	27.4%
	Breast/formula-fed	67	70.5%
	Formula-fed	2	2.1%
Antibiotic exposures[b]	Yes	62	24.7%
	No	189	75.3%
Underlying disease[b]	Yes	52	20.7%
	No	199	79.3%
Tube feeding[b]	Yes	21	8.4%
	No	230	91.6%
Surgical history[b]	Yes	34	13.5%
	No	217	86.5%
Family living together[b]	Brother/sister	172	68.5%
	Grandparents	26	10.4%
	None	72	28.7%

[a] Numbers of neonates.
[b] Numbers of non-neonatal children.

For the 251 non-neonatal children, univariate analysis was performed to screen the risk factors for *C. difficile* colonization by using the chi-square test. Both the age of 12–23 months (OR = 4.31; 95%CI [1.70–10.94]; $P = 0.002$) and tube feeding (OR = 8.22; 95%CI [3.14–21.56]; $P < 0.001$) were associated with significantly higher colonization rates of *C. difficile*. As for toxin-positive *C. difficile*, underlying disease (OR = 3.02; 95%CI [1.35–6.76]; $P = 0.005$) and tube feeding (OR = 7.46; 95%CI [2.82–19.77]; $P < 0.001$) were associated with significantly higher colonization rates (Table 2).

Multivariate logistic regression analysis was done to determine factors associated with *C. difficile* colonization, using age, antibiotic exposure, underlying disease, tube feeding, and surgical history as covariates. The age of 12–23 months (OR = 4.19; 95%CI [1.52–11.52]; $P = 0.01$) and tube feeding (OR = 24.28; 95%CI [4.70–125.34]; $P < 0.001$) were associated with significantly higher colonization rates of *C. difficile*. As for toxin-positive *C. difficile*, tube feeding (OR = 8.29; 95%CI [1.87–36.84]; $P = 0.005$) was associated with significantly higher colonization rates (Table 3).

4. Discussion

We determined the colonization rate of *C. difficile* in Japanese children. None of our neonates exhibited colonization by *C. difficile*.