Toxigenic and non-toxigenic *Clostridium difficile*: determinants of intestinal colonisation and role in childhood atopic manifestations

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and INR. MELD scores can range from 6 to 40 (MELD scores greater than 40 are all grouped together and receive a score of 40)".

Despite the fact that it should be impossible to achieve a MELD score of >40, many of the online calculators do indeed generate numbers greater than this ceiling value. This is dependent upon numbers entered for the international normalised ratio (INR), bilirubin and creatinine. We would therefore caution all clinicians when using these or similar calculators to analyse retrospective data. MELD scores can easily and incorrectly be generated with values >40.

Calculation of the median score (with interquartile ranges) would not highlight this problem, but sample mean and SD may do. These values may skew the data—for example, the 3-month survival probability with a MELD score of 40 should actually be lower than depicted on the graph, and the mean MELD score in table 1 should be <18.

This has potential ramifications for all large multicentre trials and systematic reviews utilising published MELD scores collected in this way.

We would also like to add comment to sodium not meeting the fourth criterion of "stability" in a scoring system for organ allocation. Sodium levels, like creatinine, can easily be manipulated through overzealous diuretic usage. Perhaps the measurement of variables not directly influenced by diuretic use would be of benefit.

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Toxicogenic and non-toxicogenic *Clostridium difficile*: determinants of intestinal colonisation and role in childhood atopic manifestations

In a previous paper published in this journal, we reported on the role of the intestinal microbiota in the development of atopic manifestations, as examined in a prospective birth cohort (KOALA).¹ We showed that infants colonised with *Clostridium difficile* were at increased risk of developing eczema, wheeze and sensitisation. An association between *C difficile* and atopic diseases has also been reported by others.^{2,3}

A possible underlying mechanism could be through breaking oral tolerance, as *C difficile* can cause inflammation in gut tissues,

leading to increased permeability of the mucosal barrier and thus facilitating the penetration of allergens.⁴ The enteropathogenicity of *C difficile* is associated with the production of toxins A and B, encoded by the genes *tcdA* and *tcdB*. Both toxins disrupt epithelial cell tight junctions and thereby ablate epithelial barrier function.⁵ According to the hypothesised mechanism, it is expected that especially infants colonised with toxin-producing strains are at increased risk of developing atopic diseases.

The aim of this study was to examine which factors influence (non-) toxicogenic *C difficile* colonisation and to examine the role of *C difficile* toxicogenicity on the development of atopic manifestations.

Faecal samples of 957 one-month-old infants, participating in the KOALA study, were available for analysis (for a detailed description of methods see Penders *et al*).¹ The samples have been subjected to real-time PCRs for the detection of *C difficile* and its toxins A and B, according to the assays as described in Penders *et al*,¹ Belanger *et al*⁶ and van den Berg *et al*,⁶ respectively.

Information on potential determinants of (non-)toxicogenic *C difficile* colonisation, atopic symptoms and potential confounders was retrieved through repeated questionnaires. Specific immunoglobulin E (IgE) was

measured in blood samples collected at the infant's age of 1 and 2 years. A clinical diagnosis of eczema was made at 2 years.

A total of 200 infants (20.9%) were colonised with non-toxicogenic (A[−]B[−]), 36 (3.8%) with toxicogenic A⁺B⁺ and 4 (0.4%) with toxicogenic A[−]B⁺ *C difficile*. Hospital delivery (especially caesarean section) and hospital admission following birth were associated with higher colonisation rates of both toxicogenic and non-toxicogenic *C difficile*. Exclusively breastfed infants were less often colonised with (non-)toxicogenic strains compared with their formula-fed counterparts. Boys were at increased risk of being colonised with toxicogenic strains (fig 1). Maternal education, maternal organic and/or vegetarian diet, maternal probiotic and antibiotic use during pregnancy, birth season, number of siblings, fever in the first month of life and the presence of furry pets were not associated with colonisation by either non-toxicogenic or toxicogenic *C difficile*.

Colonisation of infants with non-toxicogenic, but not with toxicogenic, *C difficile* increased the risk of developing (parentally reported and clinically diagnosed) eczema and sensitisation to food allergens (tables 1 and 2). This implies that toxins A and B are not responsible for the increased risk of eczema and sensitisation among carriers of *C difficile*. The different

Figure 1 Faecal counts of *C difficile* in infants colonised with non-toxicogenic (median 4.68 log₁₀ colony-forming units (CFU)/g; 95% range 2.73–8.23) and toxicogenic (median 7.55 log₁₀ CFU/g; range 3.0–8.41) strains. ‡Reference category

Table 1 Association between colonisation with (non-)toxicogenic *C difficile* at 1 month of age and atopic manifestation at 2 years of age (n = 957)

	Parentally reported eczema†		Clinically diagnosed eczema (UK-WP)‡		Recurrent wheeze†	
	Prevalence (n/N§)	ORadj (95% CI)¶	Prevalence (n/N§)	ORadj (95% CI)¶	Prevalence (n/N§)	ORadj (95% CI)¶
<i>C difficile</i>						
Absent	30.3% (213/702)	1.0	14.1% (64/454)	1.0	9.4% (63/671)	1.0
Non-toxicogenic	40.1% (79/197)	1.45* (1.04 to 2.03)	23.7% (28/118)	1.83* (1.10 to 3.04)	13.0% (25/192)	1.48 (0.88 to 2.47)
Toxicogenic	31.6% (12/38)	1.13 (0.55 to 2.31)	17.1% (6/35)	1.40 (0.55 to 3.58)	21.6 (8/37)	3.92** (1.62 to 9.44)

*p<0.05; **p<0.005.

†Based on parental reports in 7, 12 and/or 24 months questionnaires.

‡As determined by trained nurses during home-visits (according to UK Working Party criteria (UK-WP)).

§Numbers in the table do not always add up to 957 due to missing outcome data.

¶Adjusted ORs (ORadj) and 95% CI from logistic regression analysis: adjusted for subcohort, parental history of atopy, siblings history of atopy, age at collection of faecal sample and infant's gender.

Table 2 Association between colonisation with (non-)toxicogenic *C difficile* at 1 month of age and atopic sensitisation at 1 (n = 613) and 2 (n = 583) years of age

	Sensitisation to food allergens age 1 year†		Sensitisation to food allergens age 2 years†		Sensitisation to aeroallergens age 2 years‡	
	Prevalence (n/N§)	ORadj (95% CI)¶	Prevalence (n/N)	ORadj (95%CI)¶	Prevalence (n/N§)	ORadj (95%CI)¶
<i>C difficile</i>						
Absent	13.3% (61/460)	1.0	19.5% (86/440)	1.0	13.2% (49/370)	1.0
Non-toxicogenic	22.3% (27/121)	1.91* (1.14 to 3.18)	30.3% (33/109)	1.85* (1.14 to 3.00)	15.8% (15/95)	1.16 (0.61 to 2.19)
Toxicogenic	15.6% (5/32)	1.11 (0.4 to 3.05)	20.6 (7/34)	1.08 (0.45 to 2.62)	14.8% (4/27)	1.08 (0.35 to 3.31)

* p<0.05; **p<0.005.

†Specific immunoglobulin E antibodies to at least one food allergen (cow's milk, hen's egg, peanut), cut-off values 0.35 IU/ml (age 1 year) and 0.3 IU/ml (age 2 years).

‡Specific immunoglobulin E antibodies to at least one aeroallergen (birch, grass pollen, cat, dog, house dust mite), cut-off value 0.3 IU/ml.

§Numbers in the table do not add up to 583 due to missing outcome data.

¶From logistic regression analysis: adjusted for subcohort, parental history of atopy, siblings history of atopy, age at collection faecal sample and infant's gender.

associations of non-toxicogenic and toxicogenic *C difficile* with these atopic outcomes suggest that other bacterial components or properties, differing between toxin-positive and toxin-negative strains, may play a role.

In contrast to eczema and sensitisation to food allergens, recurrent wheeze was positively associated with toxin-positive *C difficile* (table 1). This could indicate different biological mechanisms underlying the aetiology of wheeze compared with eczema and sensitisation.

In conclusion, the present study showed that although both non-toxicogenic and toxicogenic *C difficile* strains share the same environmental origin, they are differently associated with atopic manifestations. In contradiction of our hypothesis, the association between *C difficile* and eczema and sensitisation cannot be explained by disruption of the epithelial barrier function by toxins A and B. The different associations between non-toxicogenic and toxicogenic *C difficile* with atopic manifestations, however, demonstrate the importance of studying the effect of gut bacteria in health and disease beyond the genus and species level. Further investigations are necessary to understand better the role of non-toxicogenic *C difficile* strains in disease development.

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Patient consent: All parents signed informed consent for the study.

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Upper gastrointestinal symptoms and asthma: a manifestation of allergy?

We welcomed the systematic review by Havemann *et al* (*Gut* 2007;56:1654) further highlighting the significance of the association between gastro-oesophageal reflux disease (GORD) and asthma. The importance of this association is underlined by the magnitude of published studies concerning this subject (731 studies evaluated in Havemann's review). The reviewers conceded that the direction of causality and the underlying mechanism responsible for this association remain undetermined. Mechanisms postulated include hyperexpansion of the asthmatic chest leading to failure of the gastro-oesophageal junction (GOJ) with attendant reflux symptoms, or shared stimulation of vagus nerve-mediated reflex arcs resulting in both bronchial smooth muscle constriction (causing asthma) and GOJ relaxation (causing GORD). Causality has also been speculated to occur in the reverse direction, with aspiration of gastric fluid into the bronchial tree of patients with GORD leading to symptomatic bronchial irritability and asthma.

An alternative explanation also merits consideration. Asthma is characterised by increased numbers of eosinophils in the bronchial mucosa, airways lumen and circulating blood, in numbers that correlate with asthma severity.¹ It appears that in patients with allergic diseases such as asthma and allergic rhinitis, the migration