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Does saline enema during the first stage of labour reduce the incidence of *Clostridium difficile* colonization in neonates? A randomized controlled trial

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SUMMARY

Background: Maternal rectal enemas may reduce neonatal bacterial exposure during labour, which may reduce the risk of neonatal colonization with *Clostridium difficile*. The aim of this study was to determine the effectiveness of a saline enema during the first stage of labour in reducing neonatal colonization with *C. difficile*.

Methods: This study was conducted at Cairo University Hospital, Egypt from January 2016 to July 2016. Asymptomatic mothers with uncomplicated vaginal delivery and their neonates without diarrhoea were included. The study group underwent saline enema, and the control group had no intervention. Stool samples were collected from neonates one week after delivery. The primary outcome was the detection of *C. difficile* in stool culture and direct detection of *C. difficile* Toxin A and Toxin B by enzyme-linked immunosorbent assay.

Findings: The two groups were comparable ($P>0.05$) in terms of age, gravidity, parity, body mass index and gestational age. *C. difficile* was detected in 13.54% and 37.63% of stool cultures from the enema group and the control group, respectively ($P<0.001$). Direct detection of Toxins A and B was positive in 22.92% of cases in the enema group and 53.76% of cases in the control group ($P<0.001$).

Conclusion: This study suggests that a saline enema for the mother during the first stage of labour may be useful in reducing the risk of neonatal gut colonization by *C. difficile*.

Introduction

Worldwide, the incidence of *Clostridium difficile* infection (CDI) is increasing. Hospitalization has been recognized as a risk factor for CDI by many studies [1–3]. Despite the long-held opinion that children are asymptomatic carriers of *C. difficile*, researchers have suggested recently that the bacterium is a leading cause of diarrhoea in this age group [4]. One recent study stated that CDI occurred in 26% of infants aged under one year and in 5% of neonates [5].

Colonization of *C. difficile* commonly occurs in the first week of life; the reported prevalence of the bacterium in neonates has varied widely from 2% to 50%. By two years of age, the

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rate of colonization is equivalent to that in adults. Moreover, infants colonized asymptomatically represent a potential reservoir for transmission to other members of the family [6].

The primary mode of transmission of *C. difficile* is person-to-person via the faecal–oral route, primarily in hospitalized patients where the hands of healthcare personnel are important vectors [7].

C. difficile may be found as part of the vaginal microbiota during pregnancy [8]. Exposure of the neonate at birth to vaginal, faecal and skin micro-organisms is the primary cause of neonatal bacterial colonization [9–11]. However, postnatal *C. difficile* colonization also occurs in neonates delivered by caesarean section (CS). Thus, intrapartum transmission does not appear to be the only means of acquisition of *C. difficile* by neonates [12–15]. As well as the maternal vagina, the environment may be an important source of neonatal acquisition of *C. difficile* [16]. This would be consistent with the observation from previous studies that putative risk factors, such as mode of delivery, preterm rupture of membranes, gender and the use of antibiotics for the mother or the neonate, had no impact on *C. difficile* neonatal carriage rates. In contrast, longer duration of hospital stay did appear to be associated with an increased rate of carriage [17].

Efforts to prevent CDI, especially in healthcare settings, are vital [18]. The primary objective of this randomized controlled study was to investigate the impact of using saline enema in the active phase of the first stage of labour in reducing neonatal colonization with *C. difficile*.

Methods

This study was conducted in the Obstetrics and Gynaecology Department and the Paediatrics Department, Cairo University Hospital, Egypt from late January 2016 to July 2016. This study followed the principles of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act, and was approved by the Medical Ethical Review Committee of Cairo University on 12th January 2016 (Registration No. OG-3-12-1-2016). The purpose of this study was explained clearly in Arabic to all subjects before their enrolment into the study, and an informed consent form was signed by and obtained from all of those enrolled.

Patients

Women eligible for vaginal delivery with a singleton, term baby were recruited into this study. The inclusion criteria were: asymptomatic mother with no diarrhoea, maternal age 20–40 years, and maternal body mass index 20–35 kg/m^2. Maternal exclusion criteria were: preterm rupture of membranes, complicated or instrumental delivery, CS and prematurity. Neonatal exclusion criteria were: administration of medication, neonatal intensive care unit admission, any surgical condition and diarrhoea.

All mothers and neonates were discharged home shortly after delivery (6 h post partum), and all babies were breast fed.

Randomization and blinding

Before the trial, computer-generated randomization schedules were generated and placed in sequentially numbered sealed opaque envelopes. Block randomization with a block size of four was used with a 1:1 ratio of the two groups. The allocation sequence was concealed from the researchers enrolling and assessing participants. The laboratory researcher did not know the relationship between the patients' numbers and the allocation sequence.

Enema administration

Normal saline enema was administered once to mothers in the enema group. One litre of saline was administered for 30 min. Nurses administering the enema washed their hands with soap and water, followed by decontamination with chlorhexidine. They wore disposable gloves and aprons, and a separate disposable enema set was used for each patient. Special care was taken in cleaning the bathroom after enema administration; surfaces were decontaminated using 10% sodium hypochlorite. The study and control groups were cared for in different areas of the ward, but patients were not isolated individually.

Stool sample collection

Stool samples were collected from neonates at one week of age. Before collection, the anal area was washed and a urine bag was applied to prevent contamination with urine. The individuals collecting these stool samples were blinded regarding the allocation of subjects.

Laboratory investigations

Three culture methods were used for *C. difficile*: alcohol shock followed by inoculation supplemented with 7% horse blood on to blood agar; direct culture on selective cycloserine-cefoxitin fructose agar (Oxoid, Basingstoke, UK); and enrichment culture in cycloserine-cefoxitin fructose broth supplemented with 0.1% sodium taurocholate. *C. difficile* was confirmed biochemically in colonies morphologically suspected as being *C. difficile*. Direct detection of Toxin A and Toxin B from stool samples by enzyme-linked immunosorbent assay (ELISA) was performed using the RIDASCREEN *C. difficile* Toxin A/B ELISA (C0801) (Clinilab, Cairo, Egypt), according to the manufacturer's instructions.

Statistical analysis

A sample size calculation was made to calculate the number of subjects needed in each group. It was assumed that use of the enema would reduce the incidence of *C. difficile* by at least 20%. With a significance level of 0.05 and 90% power, a minimum of 130 patients (65 patients per group) was required; as such, the target sample size was 180 to allow for dropouts.

All statistical tests were performed using a significance level of 95%. $P<0.05$ was considered to indicate significance. Statistical Package for the Social Sciences Version 20.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. Data are presented as mean ± standard deviation or median (range) for continuous and ordinal variables, and as frequency and percentage for categorical variables. Comparisons between groups were made using Student's *t*-test, the non-parametric Mann–Whitney test and analysis of variance for continuous and ordinal variables, and Chi-squared test for dichotomous variables. [19].

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Results

Four hundred and thirty eligible mothers were invited to participate in the study. Of these, 120 did not consent to participate. Of the 310 consenting mothers, 121 were subsequently excluded, leaving a final sample size of 189 (Figure 1). There were 96 mothers in the enema group and 93 mothers in the control group. There were no significant differences between the groups in terms of age, gravidity, parity, body mass index and gestational age (Table I). The neonates born to enema and control mothers were also comparable (Table II).

The rates of *C. difficile* positivity by at least one culture method were 13.54% and 37.63% in neonates in the enema and control groups, respectively ($P<0.001$). The absolute risk reduction (ARR) was 0.24 [95% confidence interval (CI) 0.12–0.36] and number needed to treat (NNT) was 4.15 (95% CI 2.77–8.26) (Table III). *C. difficile* toxin positivity was detected in 22.92% and 53.76% of cases in the enema and control groups, respectively ($P<0.001$). ARR was 0.31 (95% CI 0.18–0.44) and NNT was 3.24 (95% CI 2.27–5.56) (Table III). No complications occurred as a consequence of performing saline enema.

Table I
Patient characteristics of the enema and control groups

	Enema group	Control group	*P*-value
Age (years), mean (SD)	29.39 (4.97)	29.25 (5.27)	0.855
Gravidity, median (range)	2 (1–4)	2 (1–4)	0.588
Parity, median (range)	1 (1–4)	1 (1–4)	0.418
Body mass index (kg/m^2)	29.42 (2.75)	28.89 (2.85)	0.531
Gestational age (weeks), mean (SD)	39.39 (1.06)	39.45 (1.08)	0.685

SD, standard deviation.

Table II
Neonatal characteristics in both study groups

	Enema group	Control group	*P*-value
Neonatal temperature (°C), mean (SD)	36.84 (0.30)	36.77 (0.35)	0.126
Neonatal weight (kg), mean (SD)	3.16 (0.34)	3.11 (0.30)	0.269

SD, standard deviation.

Discussion

The results of this study show that administering a saline enema to the mother during labour could be a useful method to reduce the colonization of neonates by *C. difficile* in the first week of life. The proposed explanation for this is a reduction in the *C. difficile* load in the maternal gut, thus reducing the risk of soiling during labour, so the baby is less likely to be exposed to *C. difficile*. The use of enemas has proved to be beneficial in some conditions such as hepatic encephalopathy, where they are used as a means of expelling the ammonia-producing gut flora by cleansing and colonic acidification [20]. It was against this background that the authors decided to evaluate the use of saline enemas in the first stage of labour to reduce neonatal gut colonization with *C. difficile*.

To the authors' knowledge, this is the first study to address the question of whether saline enemas during labour could protect neonates from colonization with *C. difficile*. If these results are confirmed by others, administration of a saline enema could be a simple, non-expensive method to reduce the carrier rate of *C. difficile* in neonates. This may protect them from future diarrhoeal illness, and may even assist with controlling the spread of *C. difficile* in hospitals.

A strength of this study is its design, which was based on a randomized controlled trial with an adequate sample size. One major limitation is that *C. difficile* carriage in mothers was not investigated; this is a potential confounder because the possibility that the differences in neonatal rates simply reflected differences in maternal carriage rates cannot be excluded. However, given that the mothers were closely matched

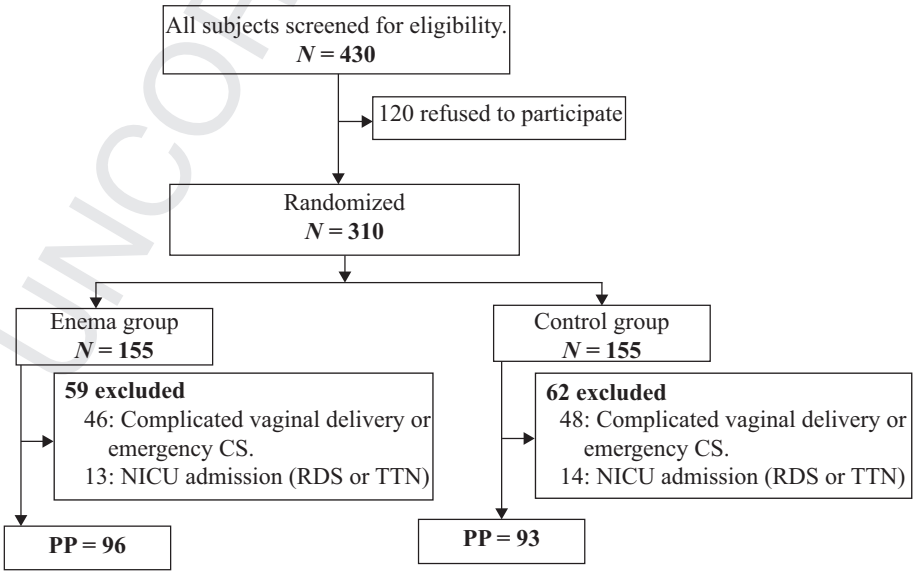

Figure 1. CONSORT diagram. CS, caesarean section; NICU, neonatal intensive care unit; RDS, respiratory distress syndrome; TTN, transient tachypnea of the newborn.

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Table III
Laboratory studies for *Clostridium difficile* in neonates in both study groups after one week

	Enema group	Control group	*P*-value
Stool culture using *C. difficile* selective medium			
Positive test	13 (13.54)	35 (37.63)	<0.001
Negative test	83 (86.46)	58 (62.37)	
Experimental event rate	0.1354		
Control event rate	0.3763		
Absolute risk reduction	0.24 (0.12–0.36)		
Relative risk reduction	0.64 (0.36–0.80)		
Number needed to treat	4.15 (2.77–8.26)		
Direct detection of *C. difficile* Toxins A and B by ELISA			
Positive test	22 (22.92)	50 (53.76)	<0.001
Negative test	74 (77.08)	43 (46.24)	
Experimental event rate	0.23		
Control event rate	0.54		
Absolute risk reduction	0.31 (0.18–0.44)		
Relative risk reduction	0.57 (0.36–0.72)		
Number needed to treat	3.24 (2.27–5.56)		

ELISA, enzyme-linked immunosorbent assay.

demographically, it is considered unlikely that there would have been a significant difference in *C. difficile* carriage rates between the enema and control groups.

In conclusion, this small, single-centre, randomized controlled trial suggests that administering saline enemas to mothers during labour has potential as a low-cost and useful method for preventing neonatal colonization with *C. difficile*.

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Conflict of interest statement
None declared.

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