PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effect of early directed implementation of family-integrated care measures on colonisation with Enterobacteriaceae in preterm neonates in NICU
AUTHORS	Parm, Ülle Tiit-Vesingi, Annika Soeorg, Hiie Štšepetova, Jelena Truusalu, Kai Vorobjov, Sigrid Lutsar, Irja Metsvaht, Tuuli

VERSION 1 - REVIEW

REVIEWER	Reviewer Name: Vishnu Bhat Institution and Country: Jawaharlal Institute of Post Graduate Medical Education, India
REVIEW RETURNED	22-Oct-2022

GENERAL COMMENTS	1. All the authors appear to be microbiologists. Participation of Neonatologists would have been better 2.First letter of 'Key words' are usually given in Capitals 3.It would have been better if the two groups belonged to same time period. There could have been other factors affecting the outcome 4.Figure.1 is not clear 5. Infants were included up to one month of life. I could not find the time of recruitment after birth. How many infants were outborn? 6. There were 14 infants below 28 weeks of gestation. How did the authors mange to give early FCC among them?

REVIEWER	Reviewer Name: Dr. Conrad Kabali Institution and Country: Canada
REVIEW RETURNED	30-Oct-2022

GENERAL COMMENTS	General comment: Too many abbreviations which makes it difficult to read the manuscript. Can you try to reduce the number of abbreviations. Page 8, line 137: Using a 95% alpha level is unconventional. Did you mean to say, "a two-sided alpha of 5%"? Page 8, line 156: Unclear which model is for the comparison of early development of gut colonization, and which one is for identifying risk factors associated with the emergence of HAS and ESBL-positive Enterobacteriaceae. Please make this distinction clear Page 9, line 163: This is a little confusing. The variable that measures "early development of gut colonization" forms the core of

	your hypothesis and shouldn't be removed under any circumstances. It seems like you would remove it if p>0.1? Page 9, line 169: Unclear which model is for the comparison of "routine vs early directed FCC" and which one is for identifying risk factors. Also, for the comparison model, please state what are the potential confounders for adjustment

REVIEWER	Reviewer Name: Dr. Anne Debeer Institution and Country: Katholieke Universiteit Leuven UZ Leuven, Belgium
REVIEW RETURNED	14-Nov-2022

GENERAL COMMENTS	I read with great interest the paper 'Effect of early directed implementation of family-centered care measures on colonization with Enterobacteriaceae in preterm neonates in NICU' Parm and co-authors. They describe the effect of early directed family centered care on colonization patterns in preterm infants. Scientific evidence for the effect of different aspects of FCC is still scarce and necessary so the authors have to be congratulated for their efforts. Abstract Results: I would keep the same order when reporting the result (routine care vs FCC) Background No comments Methods What were contra indications for MOBM – please clarify Results Table 1 amount of MOBM in ml – consider ml/kg/day Line 227-239 in legend of figure rather than in text Discussion and conclusions no comments

VERSION 1 – AUTHOR RESPONSE

Editor in Chief Comments to Author :

1. Discussion delete the 1st sentence. Journal policy is for authors to avoid describing their study as the first.

Response: Thank you. We deleted the first sentence from the study. "To the best of our knowledge, this is the first study demonstrating the effects of early directed implementation of FCC measures in NICU on early colonization with Enterobacteriaceae, including HAS and ESBL-positive strains."

2. Add Key messages (What is already known, What this study adds, etc) See Instructions to authors

Response: We included key messages after Abstract according to "Instructions for authors; Submission guidelines" (Line 71-80):

What is already known on this topic: Preterm neonates admitted to NICU show low diversity of early gut colonization compared to healthy term infants and often harbor hospital acquired strains carrying higher risk of invasive infections.

What this study adds: Early implementation of family-centered care measures may increase the diversity and reduce colonization with hospital acquired strains of Enterobacteriaceae.

How this study might affect research, practice or policy: Data on the effects of family centered neonatal intensive care measures on early neonatal colonization, which has been shown to have long-term health impacts, are limited. Further well-designed large studies are needed to allow identification and implementation of best practices.

3. Abstract add details that it is a before and after study comparing two different time periods

Be cautious in your conclusions

Response: Thank you for this comment. We have clarified the study design and rephrased the conclusion in the abstract as follows:

In the method section the following explanation has been added (Line 110-115): “During the first period, neonates were admitted to open bay unit with later transfer to single family rooms based if available; feeding with mother’s own breast milk (MOBM) was introduced within 24 hours and skin-to-skin contact (SSC) within 5 days of life (routine care group). During the second period, following a wash-in of two months, care in single family room within 48 h, introduction of MOBM within two and SSC in 48 h were applied (intervention group).”

In the results section the following information has been added (Line 190-194): “In the intervention group, SSC and MOBM feeding was started significantly earlier; during the first week of life, time spent in SSC was longer (median h per day 4.8 (4-5.1) vs 1.9 (1.4-2.6), p<0.001) and the proportion of MOBM in enteral feeds was higher (median (IQR) 97.8% (95.1-100) vs 95.1% (87.2-97.4), p=0.011)”.

The conclusion has been rephrased as follows (Line 347-348): “Early implementation of FCC measures may hold the potential to increase diversity and reduce colonization with Enterobacteriaceae HAS.”

Reviewer: 1

Vishnu Bhat, Jawaharlal Institute of Post Graduate Medical Education

1. All the authors appear to be microbiologists. Participation of Neonatologists would have been better

Response: We are sorry if the authors’ affiliations statement has been somehow misleading. Two of our authors are actually neonatologists (Annika Tiit-Vesingi and Tuuli Metsvaht) with over 20 years of experience in the field. Both are also currently working in NICU. For the sake of clarity, we have changed the order of their affiliations in the manuscript.

2. First letter of 'Key words' are usually given in Capitals

Response: Thank you, we have corrected this error.

3. It would have been better if the two groups belonged to same time period. There could have been other factors affecting the outcome

Response: We agree that parallel groups would have been preferable. Unfortunately, due to the nature of the study intervention, we believed it would not be feasible to carry on the two approaches simultaneously within one unit. We believe, that this would have had huge likelihood of contaminating the “routine care” approach. For the same reason, we could not apply random selection of the

periods' order. It is certainly one of the limitations of our study as has been stated in the section of limitations in the manuscript:

4.Figure.1 is not clear

Response: Thank you for pointing to this misleading indistinctness. We shifted the following text from chapter "Colonization with HAS and ESBL-positive strains" into the information in figure legend (Line 247-261). The relocated text is presented in red.

Figure 1

"Overtime spread by family unit (panel A) and measured and modelled overall proportion (panel B) of hospital acquired strains of Enterobacteriaceae during the two study periods".

In panel A the Y-axis shows PFGE-type and X-axis shows study period (time from birth of the first newborn included in the study till the last newborn concluded the study). Family units harboring hospital acquired strain(s) of the specified Enterobacteriaceae species are marked by black and all other harboring at least one non-hospital strain of the specified Enterobacteriaceae species by grey dots. Vertical dotted lines between two periods indicate interim period. Size of the node is proportional to the number of isolates within the family group. In panel B, the actual proportion of hospital strains among all Enterobacteriaceae isolates by month is shown in black dots and line. Grey line and dots represent proportion of hospital acquired strains as predicted by autoregressive integrated moving average (ARIMA) model. The dotted lines between two periods represents interim period. Note that the discontinuities of the lines are due to the non-availability of the data on the proportion of the hospital strains and study intervention during the interim period.

5. Infants were included up to one month of life. I could not find the time of recruitment after birth. How many infants were outborn?

Response: We apologise, if the methods section has not been sufficiently clear/ specific on the time of recruitment. All neonates were born in the same centre and recruited as soon as possible, most of them within the first few hours, after birth. The study period, during which they were followed-up lasted for one month.

The text has been revised as follows (Line 116-117): "We included inborn neonates with gestational age (GA) below 34 full weeks and their parents as soon as possible after birth if"

6. There were 14 infants below 28 weeks of gestation. How did the authors mange to give early FCC among them?

Response: There are several reasons we managed to perform early FCC. The most important fact is the mind-set of East-Tallinn Central Hospital's NICU healthcare professionals. Both parents' presence as caregivers of their baby in NICU is very common in Estonia. We see parents as primary caregivers and involve them in the care for their infant. SSC is routine practice and personnel is trained to encourage both parents independent of the premature baby's gestational age. Therapeutic interventions like need for respiratory support, central venous and arterial lines in place are not considered a contraindication for SSC. The unit has developed routines of SSC to avoid cooling in extremely preterm infants. It has been shown in previous studies, that close contact with the baby promotes breastmilk expression and exclusive breast feeding.

We must also admit, that during the informed consent process we explained the principles of care and included only parents, who were ready to provide care in accordance with our directed FCC

principles. However, the number of parents-baby diads/triads, who refused participation was very low – only one family declined participation in the study.

Reviewer 2

Dr. Conrad Kabali

1. General comment: Too many abbreviations which makes it difficult to read the manuscript. Can you try to reduce the number of abbreviations.

Response: Thank you for drawing attention to this issue. We have removed the following less widely used abbreviations from the text and also from list of abbreviations: CSV (central venous catheter), UAC (umbilical arterial catheter), PN (parenteral nutrition), OBU (open bay unit), single family room (SFR), routine care group (RCG) and intervention group (IG)

2. Page 8, line 137: Using a 95% alpha level is unconventional. Did you mean to say, "a two-sided alpha of 5%"?

Response: Thank you for pointing out this error. The text has been revised and now reads as follows (Line 154-155): “With a two-sided alpha of 5% and power of 80% to detect …”

3. Page 8, line 156: Unclear which model is for the comparison of early development of gut colonization, and which one is for identifying risk factors associated with the emergence of HAS and ESBL-positive Enterobacteriaceae. Please make this distinction clear

Response: We apologise for the unclear wording of this part of the text. Univariate logistic regression models were used to determine potential risk factors of 1) cumulative colonisation with hospital acquired Enterobacteriaceae strains and 2) cumulative colonisation with ESBL positive Enterobacteriaceae strains. The routine cre group and the intervention group were compared only by t-test or Mann-Whitney test (continuous variables) and Chi-square or Fisher exact test (categorical variables).

The text has been rephrased as follows:

Line 160-162: "The routine care group and the intervention group were compared by t-test or Mann-Whitney test (continuous variables) and Chi-square or Fisher exact test (categorical variables)". Line 163-170: “Univariate logistic regression models were applied to determine risk factors of cumulative colonization of any studied site (including neonatal gut, MOBM and parents’ skin) with 1) hospital acquired and 2) ESBL strains of Enterobacteriaceae by week 4. within a family group. The following independent variables were included: study group, gestational age, mode of delivery, presence of PROM, maternal antibiotics, time of first MOBM and proportion of MOBM in enteral feeds, time of first and total duration of SSC during NICU stay, duration of hospitalization, use and duration of central venous and umbilical arterial catheter and unit personnel contact time with newborn..”

4. Page 9, line 163: This is a little confusing. The variable that measures "early development of gut colonization" forms the core of your hypothesis and shouldn’t be removed under any circumstances. It seems like you would remove it if p>0.1?

Response: We apologise for the unclear wording. As we understand, this question is part of the misunderstanding, mentioned above. We have clarified the issue above. The process of multivariate logistic model development was performed as described in the manuscript.

We removed all variables from multivariable models “until only variables with p-value <0.05 were retained” (Line 173). We totally agree that retaining variables that affect early gut colonization

according to causal relationships is necessary to test hypotheses about the effect of the intervention on dependent variable. As our aim was “to identify risk factors associated with the emergence of HAS and ESBL-positive Enterobacteriaceae” (Lines 104-105), we believe that descriptive modelling to capture the association between the dependent and independent variables is appropriate. Although it would be relevant to test the effect of the intervention, explanatory modelling would have required appropriate sample size. However, the number of colonized neonates was small (24 with HAS, 8 with ESBL-positive Enterobacteriaceae), and thus, proper explanatory modelling was unattainable.

5. Page 9, line 169: Unclear which model is for the comparison of “routine vs early directed FCC” and which one is for identifying risk factors. Also, for the comparison model, please state what are the potential confounders for adjustment

Response: Logistic regression models were used to identify risk factors. The routine care group and the intervention group were compared only by t-test or Mann-Whitney test (continuous variables) and Chi-square or Fisher exact test (categorical variables), as explained above. As these test only association between two variables, no adjustment for confounders was performed. Further, „ The proportion of HAS among all isolates was analysed as time series data using autoregressive integrated moving average (ARIMA) with study group as an exogenous variable“.

Reviewer: 3

Dr. Anne Debeer, Katholieke Universiteit Leuven UZ Leuven

1. I read with great interest the paper 'Effect of early directed implementation of family-centered care measures on colonization with Enterobacteriaceae in preterm neonates in NICU' Parm and co-authors. They describe the effect of early directed family centered care on colonization patterns in preterm infants.

Scientific evidence for the effect of different aspects of FCC is still scarce and necessary so the authors have to be congratulated for their efforts.

Response: Thank you.

2. Abstract. Results: I would keep the same order when reporting the result (routine care vs FCC)

Response: Thank you, we have rephrased the sentence as follows (Line 65-67): “Compared with the routine care group, the intervention group showed higher SID and reduction of HAS by 33.1% (95% CI 24.4-42.4%) in time series analysis ”.

3. Background. No comments

Response: Thank you.

4. Methods. What were contra indications for MOBM – please clarify

Response: The following clarification has been added in the methods section (Line 120-121): “(1) had contraindications to MOBM (e.g. known HIV positive status)”

5. Results. Table 1 amount of MOBM in ml – consider ml/kg/day

Response: We agree, that in most cases ml/kg/die would allow a better overview. However, given the relatively narrow weight range and also changing body weight of very preterm neonates over the first weeks of life, we felt that reporting the volumes in ml/die will be adequate.

6. Line 227-239 in legend of figure rather than in text

Response: We absolutely agree. We agree, that Figures must be clear. So we deleted the text from chapter “Colonization with HAS and ESBL-positive strains” and for better understanding added the information in figure legend (Line 247-261). The changed text is presented in red here.

Figure 1

"Overtime spread by family unit (panel A) and measured and modelled overall proportion (panel B) of hospital acquired strains of Enterobacteriaceae during the two study periods”.

In panel A the Y-axis shows PFGE-type and X-axis shows study period (time from birth of the first newborn included in the study till the last newborn concluded the study). Family units harboring hospital acquired strain(s) of the specified Enterobacteriaceae species are marked by black and all other harboring at least one non-hospital strain of the specified Enterobacteriaceae species by grey dots. Vertical dotted lines between two periods indicate interim period. Size of the node is proportional to the number of isolates within the family group. In panel B, the actual proportion of hospital strains among all Enterobacteriaceae isolates by month is shown in black dots and line. Grey line and dots represent proportion of hospital acquired strains as predicted by autoregressive integrated moving average (ARIMA) model. The dotted lines between two periods represents interim period. Note that the discontinuities of the lines are due to the non-availability of the data on the proportion of the hospital strains and study intervention during the interim period.

7. Discussion and conclusions no comments

Response: Thank you.