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Missing **M**icrobes in **I**nfants Born by **C**aesarean Section: Antenatal Antibiotics and Mode of Delivery

(MiMIC Study)

Supplement 1 – Informed Consent Form (ICF)

Parent Information Leaflet

Dear Mother,

You are being asked to take part in a research study currently being undertaken in the Department of Neonatology, Cork University Maternity Hospital and in collaboration with APC Microbiome Ireland (University College Cork, Teagasc Food Research Moorepark) and International Flavors & Fragrances. In order to decide whether or not you want to take part, we would like you to know some more about the study.

This parent information leaflet provides you with that information about the research study. Once you understand the study, you will be asked to sign this form if you wish to take part.

Please see data protection notice for information regarding your rights and how your data will be treated. The investigator will go through the data protection notice and how it is relevant to you.

These information leaflets are for you to keep.

What is the study about?

The gut microbiota (community of bacteria, fungi and viruses) plays a major role in early development of infant health, they impact on the developing immune system, help protect against infections, and influence how chemical processes occur in the infant's body. Infants obtain the microbiota in a few separate ways including through the birth canal, skin to skin contact between mother and through breast feeding. Breast milk, a natural prebiotic source provides the best active ingredients for the growth of beneficial microbiota (good bacteria) species.

In this study, we wish to investigate the microbiota that are present in mothers antenatally and how mode of birth, through breast milk and antibiotic treatment to either mother or infant can influence the microbiota which develop in the newborn infant.

We would like to isolate the microbiota and associated bioactive compounds which are present in the samples that may restore overall gut health to infants where their microbiota has been disturbed. We would also like to use this isolated microbiota in our preclinical study, in which we will evaluate the effect of the isolate in the animal model of C-section. This would be of benefit for future infant nutrition which if given may help treat or prevent microbial disturbances in the digestive system and will have long term health benefits.

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What is my involvement in the study?

We wish to recruit 500 expectant mothers who wish to breast feed their infants and deliver at 35 weeks plus gestation.

You Will Give:	**Your Infant Will Give:**
• Stool sample during the 3rd trimester of your pregnancy, at birth (+1 day) and 4 weeks post birth. • Breast milk samples 1 week, 4 weeks, 8 weeks and 24 weeks, 1 year, 18 months and 2 years post birth or **for as long as you are breast feeding**. • Vaginal sample if you delivery naturally. • Oral saliva sample at 8 weeks post birth. • Hair sample at 4 weeks post birth. • Mental Health questionnaires 3rd trimester and 8 weeks post birth. • Complete a food frequency questionnaire at 3rd trimester and 24 weeks post birth.	• A stool (poo) sample at 1 week, 4 weeks, 8 weeks and 24 weeks, 12 months, 18 months and 24 months to check the microbiota which are present. • Hair Sample at 1 week (if adequate hair) to check cortisol levels. • Urine sample at 4 weeks to check metabolomics. • A saliva sample at 8 weeks to check cortisol levels. • Neurodevelopmental test (Bayleys) at 2 years of age. The Bayley scales of infant and toddler development is an assessment designed to measure physical, motor, sensory, and cognitive development in babies and young children.

The samples will be collected either in the hospital while you are an inpatient otherwise, we will send you a reminder about collecting the sample and collect it from your home.

We would also like to access your records with your GP to see if you have attended for treatment for infections such as UTI (urinary tract infections) or coughs and colds and if you or your infant were prescribed antibiotics. The reason for this is to see if their use impacts the microbiota that develop in you or your infant. We would also like to contact your GP (with your permission) if in the event that the questionnaire you complete regarding the Edinburgh Depression Scale identifies antenatal or postnatal depressive symptoms.

The results of this project will provide new information on microbiota (healthy bacteria) which will help to better understand the role that these bacteria play in the health on the infant's development and if we can begin to develop a probiotic that will help restore depleted good microbiota.

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Will my or my baby's identity be revealed?

No. All information will be stored securely and will be treated with the strictest confidence. We would like your permission to show this information to other medical professionals for research and teaching purposes on occasion but will not reveal any identifying details about you or your baby.

Do I have to take part in this study?

You do not have to take part in this study, if you do not wish to. If you decide to take part, you may withdraw at any time without having to give a reason. Your decision on whether to take part, will not affect the care and management of your baby in any way. If you decide to take part, you will be given a copy of this information sheet to keep and be asked to sign a consent form, a copy of which you will also be given to keep.

What happens if I start the study and change my mind later?

You do not have to take part in the study, participation is entirely voluntary. Refusal to participate, or discontinuing participation at any time, will involve no penalty, loss of benefits or denial of treatment or services by the Cork Teaching Hospital or the participating doctor. Samples from individuals withdrawing from the study participation will be stored and analyzed in the study.

Funding of the study

There are no cost implications for the Health Service Executive (HSE) or to you. The management of patients and investigative tests will comply with current standards of care. Cost of research tests will be incurred by APC Microbiome Ireland, University College Cork.

The study is funded by International Flavors & Fragrances.

Will my baby be followed up in the future?

We will contact you once the study is complete to let you know the results of the study.

Who should I contact if I have more questions about this study?

Do not hesitate to speak to the consultant looking after you or your baby or any of the research doctors if you need more information about this study. We will be happy to answer any questions that you may have.

After you have read this information and once you have fully understood the procedures, the doctors will ask you to sign a consent form. The doctors will discuss and issues you may have about the test.

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Contact Information

Prof Gene Dempsey: Consultant Neonatologist (021 492 0524)

Research Nurses:

Names XXXXXXXXXX, Telephone Numbers: XXXXXXXXXX, Email addresses:

XXXXXXXXXX

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CONSENT BY SUBJECT FOR PARTICIPATION

Participant's Name: _____

(please print)

Title of Protocol: Missing Microbes in Infants born by Caesarean Section (**MIMIC**)

Principal Investigator: Prof Gene Dempsey (021 492 0524),

Participation in this study is voluntary and you may withdraw at any time for any reason

The research project and procedure associated with it have been fully explained to me. All experimental procedures have been identified and no guarantees have been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary, and I may withdraw consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. I understand that the investigators have such insurance as is required by law in the event of injury resulting from this research.

I, the undersigned, hereby consent to participate as a subject in the above described project conducted at the Cork University Hospital and University College Cork. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the Doctor listed. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork.

Analyses of all samples and information collected will be conducted in the CUMH (Cork University Maternity Hospital) Teagasc and UCC. On some occasions the analyses may be done in collaboration with third parties, including commercial partners (including International Flavors & Fragrances), which may require samples to be shipped to these organisations. In addition, samples and data will be stored and may be used in other research studies. In all cases, samples and data will be coded with subject identifier numbers. (**Note**: Data protection laws outside the EU may not be as stringent as those in the EU).

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Access to samples and/or data will require approval from the Director and/or Executive Management Group of APC Microbiome Ireland, Cork.

I agree that results and microbial isolates may be used to design a commercial strategy or product for gut health.

I agree that my contact details may be made available for recruitment to further studies and also allow a follow up check on my status following my participation by this project team or its Clinical Research Ethics Committee approved collaborators.

On completion of the study you will be informed of the results of the study.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Mother's Name
(Please Print)

_____

Infant's Name

(Please Print)

_____

Mother's Signature Date

_____ _____
(DD/MM/YYYY)

Researcher's Name
(Please print)

_____

Researcher's signature Date

_____ _____
(DD/MM/YYYY)

Warda AK, *et al. BMJ Open* 2022; 12:e064398. doi: 10.1136/bmjopen-2022-064398

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ADDITIONAL *OPTIONAL* CONSENT FOR STORAGE OF BIOLOGICAL SAMPLES AND DATA FOR ANALYSIS AND USE IN FUTURE STUDIES;

In addition to use of samples for the above study, I agree that my (and my baby's) biological samples and data can be stored and may be analysed for use in future ethically approved microbiome and/or nutritional research studies. In all cases, samples and data will be coded with subject identifier numbers.

If you agree to storage of your samples and data, and those of your baby, for use in further studies conducted by APC Microbiome Ireland, or its affiliated partners, please sign below where indicated;

Mother's Name (Please Print) _____

Infant's Name (Please Print) _____

Mother's Signature _____ Date _____ (DD/MM/YYYY)

Researcher's Name (Please print) _____

Researcher's signature _____ Date _____ (DD/MM/YYYY)

Supplement 2 – Stool/Vaginal swab DNA extraction method

A 0.2-g stool sample (or the cotton part of vaginal swab) will be incubated with 1 ml RBB lysis buffer (500 mM NaCl, 50 mM tris-HCL, pH 8.0, 50 mM EDTA and 4% sodium dodecyl sulphate (SDS)) in a 2-ml screw cap tube with sterile zirconia beads (A single 2.3 mm bead, 0.1 ml scoop of 1.0 mm beads and 0.1 ml scoop of 0.1 mm beads). It will be homogenised for 3 minutes (Mini-Beadbeater™, BioSpec Products, Bartlesville, USA), followed by incubation at 70°C for 15 min to further lyse the cells. Samples will be centrifuged at 4°C for 5 min at 16,000 g, the supernatant will be transferred to a fresh tube, and the RBB steps will be repeated by adding 0.3 ml of fresh RBB lysis buffer to the once used screw cap tube with beads. The supernatants from the first and second bead-beating will be pooled and incubated on ice for 5 minutes with 350 µl of 7.5 M ammonium acetate (Sigma). Samples will be centrifuged at 4°C for 10 min at 16,000 g, the supernatant will be mixed with one volume of isopropanol to precipitate the DNA. After overnight (minimum 3 hours) incubation at 4°C samples will be centrifuged at 4°C for 15 min at 16,000 g into a nuclear pellet which will be washed with 200 µl of 70% (v/v) ethanol. The pellet will be allowed to air dry for 15 min, then re-suspended in 100 µl TE buffer (Sigma), and treated first with 2 µl of 10 mg/ml RNAse (ThermoScientific, Vilnius, Lithuania) for 15 min at 37°C and next with 15 µl Proteinase K (Qiagen) and 200 µl Buffer AL (Qiagen) for 10 min at 70°C. DNA will be cleaned with QIAamp Fast DNA Stool Mini Kit (Qiagen, UK) either manually or using QIAcube Connect (Qiagen) according to manufacturer's recommendations. Final elution will be done in 200 µl of AE buffer (Qiagen). DNA concentration will be measured using Qubit 1X dsDNA HS Assay Kit (Invitrogen) according to manufacturer's instruction. DNA will be stored at −20°C.

Supplement 3 – Milk DNA extraction method

Defrosted milk (5ml) will be centrifuged at 4°C at 5,000 g for 30 minutes, followed by supernatant decantation and centrifugation at 4°C at 13,000 g for 1 minute. All supernatant will be removed with pipette and residue/fat layer will be removed with a sterile cotton swab. Pellet will be resuspended in 1 ml of sterile Phosphate Buffered Saline (PBS; 806552, Sigma Aldrich, Wicklow, Ireland), transfer to a 1.5 ml Eppendorf tube and centrifuged at 13,000 g for 1 minute at room temperature. These wash steps will be repeated until white residue will be removed from the pellet (2-3 times). Next, 90 µl of 50 mg/ml lysozyme and 25 µl of 10 KU/ml mutanolysin will be added, vortexed and incubated at 55°C for 15 mins, followed by the addition of 28 µl proteinase K and incubation at 55°C for 15 mins. Tubes will be centrifuged at 4°C at 16,000 g for 8 mins before supernatant removal. Next, a Qiagen DNeasy Powerfood Microbial Kit will be used according to manufacturer´s instructions. The resulting DNA concentration will be measured using Qubit 1X dsDNA HS Assay Kit (Invitrogen) according to manufacturer´s instruction. DNA will be stored at −20°C.

Supplement 4 – Hair cortisol extraction method

For mothers, three 1-cm hair sections located closest to the scalp/hair root will be analysed. For infants, whole length of hair sample will be analysed. For each hair section (whole length of hair sample for infant) 10 mg of hair will be transferred into a Eppendorf tube, followed by addition of 1 ml of methanol and overnight incubation at 50°C. Next, samples will be sonicated for 30 min and placed on heated shaker for 90 min at 50°C, followed by overnight incubation at 50°C. Methanol fraction will be transferred into clean tube and evaporated to dryness using nitrogen for 90 min at 37°C. Finally, resulting residue will be resuspended in 250 µl PBS (Sigma – 806552), vortexed and stored at −80°C.

Supplement 5 – Monitoring and quality control (as extracted from Study Protocol)

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

The IEC/IRB, or an auditor authorized by the sponsor may request access to all source documents, case report forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities. Medical records and other study documents will be available during audit or inspection.

Four minimum monitoring activities will be performed: site initiation visit, two during the trial, and a close out. Clinical Research Facility Cork HRB will conduct monitoring according to the monitoring plan. This representative will source verify the case report forms for completeness and clarity. Representatives of the sponsor can co-monitor the study at their own discretion. Study close-out will be performed by the study monitor upon closure of the study.

During or after the study is completed, Sponsor or Funder representatives may wish to carry out an audit or competent authorities may conduct regulatory inspections. These representatives must have the same access to study data and participant source data as the monitor.