

BMJ Open Cohort profile: Infant Gut Bacterial Study in Pakistan (INBUGS-P) longitudinal birth cohort

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ABSTRACT

Purpose The Infant Gut Bacterial Study in Pakistan (INBUGS-P) was established to characterise the longitudinal development of the infant gut microbiome and resistome during the first year of life in a low- and middle-income country setting. The influence of early-life exposures, including mode of delivery, antibiotic use and infant feeding practices on gut bacterial diversity and antimicrobial resistance gene (ARG) profiles is being evaluated.

Participants A total of 107 mother–infant pairs were recruited at the Pakistan Institute of Medical Sciences between December 2023 and June 2024. Follow-up was conducted at 10 predefined timepoints from birth to 12 months, during which 921 infant stool samples, 158 maternal rectal swabs, 246 breast milk samples and 2171 environmental swabs were collected. Sociodemographic, clinical, cultural and biological data were collected at enrolment and at each follow-up visit using Research Electronic Data Capture.

Findings to date Baseline characteristics of 98/107 mother–infant dyads are included in the analysis. The cohort reflects an urban low-income population: median household income was PKR 30,000 per month (approximately US\$170 per capita per month). Caesarean section accounted for 55% (54/98) of deliveries; 13.0% of infants were late preterm, and 10.0% had low birth weight (<2500 g). Breastfeeding was the predominant feeding mode though only 24 infants were exclusively breastfed from birth to 6 months. Antibiotics were prescribed to almost all mothers following delivery, and 19 infants received antibiotics during follow-up, most commonly amikacin combined with ceftazidime.

Future plan Shotgun metagenomic sequencing of infant stool samples is underway to enable species-level and plasmid-level profiling of microbial communities and ARGs. Subject to funding, hybrid long- and short-read sequencing and extended follow-up to 24 months are planned.

INTRODUCTION

The human microbiome, a diverse community of microorganisms inhabiting the human body, is an important determinant of health and disease, with data suggesting that perturbations in microbial communities during the infant period may have long-lasting health

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The prospective longitudinal birth cohort design in Pakistan enables characterisation of infant gut microbiome and resistome development in an under-represented population.
- ⇒ Repeated stool sampling at predefined time points from birth to 12 months and long-read shotgun metagenomic sequencing using Oxford Nanopore technology enable characterisation of microbial colonisation, succession and antimicrobial resistance gene dynamics.
- ⇒ Comprehensive longitudinal collection of clinical, sociodemographic, socioeconomic and environmental data enables evaluation of factors associated with microbiome and antimicrobial resistance dynamics.
- ⇒ Hospital-based recruitment from a single tertiary care hospital may limit the generalisability of the findings to infants born outside tertiary care settings.
- ⇒ Follow-up is currently limited to the first year of life, restricting assessment of longer-term microbiome and resistome development; statistical power for subgroup analyses was limited for less common exposures such as infant antibiotic use.

repercussions.^{1–5} The significance of the human microbiota is paramount to the maintenance of a healthy gut by aiding in metabolism, digestion of food to release nutrients that might otherwise be unavailable to the host, and nutrient absorption,⁶ immune system maturation as well as defence against pathogens; that is, indigenous microbiota helps prevent the colonisation of the body by exogenous pathogens.^{7,8}

While acknowledging the numerous advantages of indigenous microbiota, it is essential to consider whether they may inadvertently contribute to the development of antimicrobial resistance (AMR).^{9–11} AMR, an escalating global health threat,^{12–14} is increasingly being recognised as an issue beginning at birth, shaped by maternal, environmental and clinical exposures.^{15,16} In low- and middle-income countries (LMICs), antibiotic use is often less

regulated, and perinatal healthcare settings frequently face resource and infection-control challenges.^{17–19} Together, these factors make these settings a priority for investigating early-life development of the gastrointestinal (gut) microbiome and resistome.

Pakistan ranks among the countries with high infant mortality rates (approximately 50 deaths per 1000 live births).²⁰ Infectious diseases, including neonatal sepsis, account for a substantial proportion of early-life morbidity and mortality in this setting.²¹ Previous studies demonstrate early-life factors, including birth mode, infant feeding, antibiotic exposure and geographic setting, influencing gut microbiome colonisation and resistome development during infancy.^{22–28} However, most studies are from high-income countries, with limited longitudinal data from LMICs, particularly South Asia. As a result, there is limited understanding of how differences in environmental, clinical and socioeconomic factors influence gut microbiome colonisation and antimicrobial resistance gene (ARG) patterns during early life. Furthermore, relatively few longitudinal microbiome studies have incorporated long-read shotgun metagenomic sequencing, which provides improved characterisation of

microbial composition and ARGs and facilitates linkage of ARGs to their bacterial hosts.^{29 30}

Previous research within the Burden of Antibiotic Resistance in Neonates from Developing Societies (BARNARDS) project highlighted higher carriage of bacteria with extended-spectrum β -lactamase (ESBL) and carbapenemase genes among neonates in several LMICs, including Pakistan.³¹ While the human gut microbiome is known to act as a natural reservoir of diverse ARGs, the detection of clinically significant resistance markers during the neonatal period is particularly concerning. These findings highlighted the need to better understand how ARGs are acquired and evolve alongside the developing gut microbiome, particularly in relation to early-life exposures. In response, the infant gut bacterial study in Pakistan (INBUGS-P) was established to investigate the longitudinal dynamics of the gut microbiome and ARG acquisition in this setting. A parallel study (INBUGS-N) is being conducted in Nigeria.³²

The INBUGS-P study has been established to address these gaps through a prospective, longitudinal birth cohort recruited from a major public-sector hospital in Islamabad, Pakistan. This cohort profile describes the

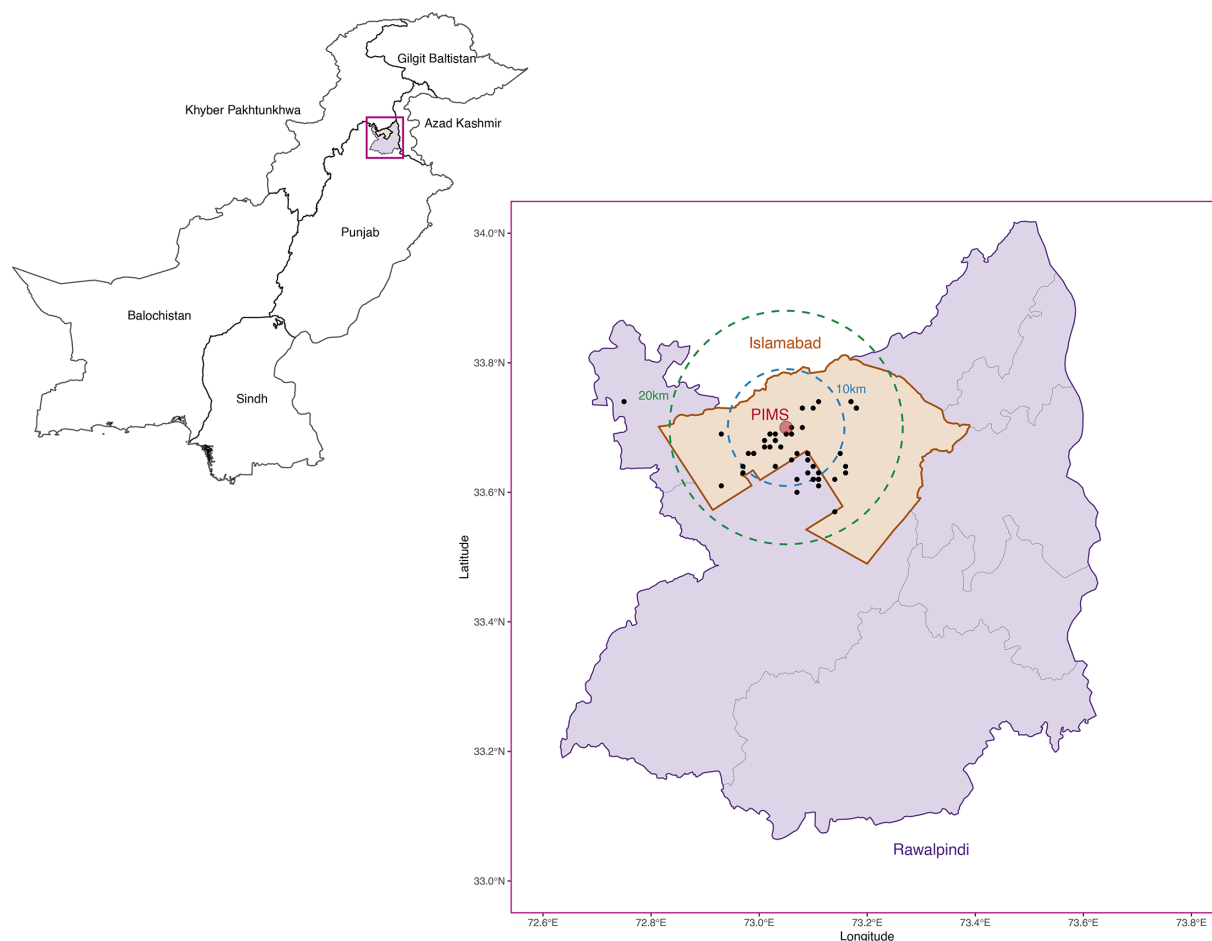


Figure 1 Geographic distribution of enrolled households in Islamabad, Pakistan. The inset shows the study area within Pakistan, with households distributed across central and outlying areas of Islamabad and neighbouring Rawalpindi. Black dots represent household locations. Dashed circles indicate 10km and 20km distances from the study site. PIMS, Pakistan Institute of Medical Sciences.

study design, recruitment and baseline characteristics to establish the basis for future longitudinal microbiome and resistome development analyses.

COHORT DESCRIPTION

Study purpose

This cohort study is designed to investigate the development and dynamics of the infant gut microbiome and resistome during the first year of life in an LMIC setting in South Asia. The study aims to (1) describe the gut bacterial diversity and its relative abundance, (2) determine the prevalence and longitudinal variation of ARGs, (3) examine the influence of early-life exposures, including mode of delivery, infant feeding and cultural practices and antibiotic use and (4) evaluate the associations between clinical, sociodemographic, socioeconomic, environmental factors and changes in the infant gut microbiome and ARG profiles.

Setting

Participants were recruited at the Pakistan Institute of Medical Sciences (PIMS), a 592-bed public tertiary care hospital in Islamabad, Pakistan, conducting over 800 deliveries per month. The cohort comprises infants born between December 2023 and June 2024. As a public referral centre, PIMS serves a socioeconomically and ethnically diverse population. Household follow-up was restricted to families residing within Islamabad (straight-line distance from PIMS: median 10.3 km (IQR 5.9–12.2), range 0.5–29.8 km) (figure 1). Enrolled households were distributed across both central and outlying neighbourhoods of the city, reflecting representation from densely populated urban sectors as well as surrounding residential areas.

Recruitment and eligibility

Mothers were approached within 24 hours of delivery by trained female research staff and invited to participate. Recruitment took place in the postnatal ward once the mother was clinically stable. The study was explained verbally in the participant's preferred language, and written informed consent was obtained prior to enrolment. Recognising the role of family involvement in decision-making in this setting, participants were given the opportunity to discuss participation with family members before providing consent. Participation was entirely voluntary. Participant information sheets outlining the study objectives, procedures and follow-up requirements were provided. In addition, informational flyers summarising key study details (online supplemental material 1) were made available at hospital reception areas and maternity units to increase awareness of the study among patients and accompanying family members.

Inclusion criteria were live-born, clinically stable infants delivered at ≥ 34 weeks' gestation, absence of major congenital anomalies and maternal residence within Islamabad city. Exclusion criteria were maternal

antibiotic use in the preceding 6 months and adherence to restrictive diets (eg, vegan, vegetarian or medically prescribed diets) to minimise potential heterogeneity in maternal dietary exposures that could influence early-life gut microbiome and resistome development.

Participant engagement and retention

To minimise attrition, eligibility was restricted to permanent residents of the city or individuals not anticipating relocation during the study period. Engagement strategies included recruitment of local research assistants to support community engagement and communication of study procedures in the local language (Urdu), assurance of confidentiality and anonymity and clarification that biological sample collection involved no invasive procedures.

To enhance retention, non-cash incentives were provided at recruitment and during follow-up visits, including baby care items. Follow-up sample collections were conducted at participants' homes to minimise participant burden. Visits were scheduled at mutually convenient times, with reminder calls made prior to appointments and any missed visits were rescheduled. These approaches facilitated sustained engagement and contributed to maintaining high retention over the first year of follow-up.

A total of 109 liveborn infants were enrolled at birth and followed at 10 predefined timepoints from birth to 12 months. Retention remained high throughout the neonatal period, with 107/109 infants (98.2%) in active follow-up by day 7 and 106/109 (97.2%) at 2 weeks; one neonatal death occurred on day 1. Retention declined gradually thereafter, with 100/109 infants (91.7%) at 1 month, 96/109 (88.1%) at 6 months and 90/109 (82.5%) at 12 months. Overall, 98 mother–infant dyads were included in the neonatal period analysis (1 month; $n=100$ infants), and 91 infants were included in the longitudinal analysis at 12 months (figure 2).

DATA COLLECTION

Questionnaire-based and clinical data were collected using Research Electronic Data Capture (REDCap). The INBUGS-P sub-study is embedded within the ongoing BARNARDS-II project, a multisite study investigating neonatal sepsis and AMR across LMICs.³³ Sociodemographic and clinical data for each mother–infant dyad were collected using standardised BARNARDS-II REDCap case report forms, supplemented by a dedicated INBUGS-P sub-study form administered at enrolment and each longitudinal follow-up visit (figure 3).

Questionnaire data

Maternal sociodemographic variables included age, parity and household income. At each longitudinal follow-up, infant feeding practices, timing of weaning, such as the introduction of water or other supplementary foods before 6 months of age and traditional pre-lacteal feeding

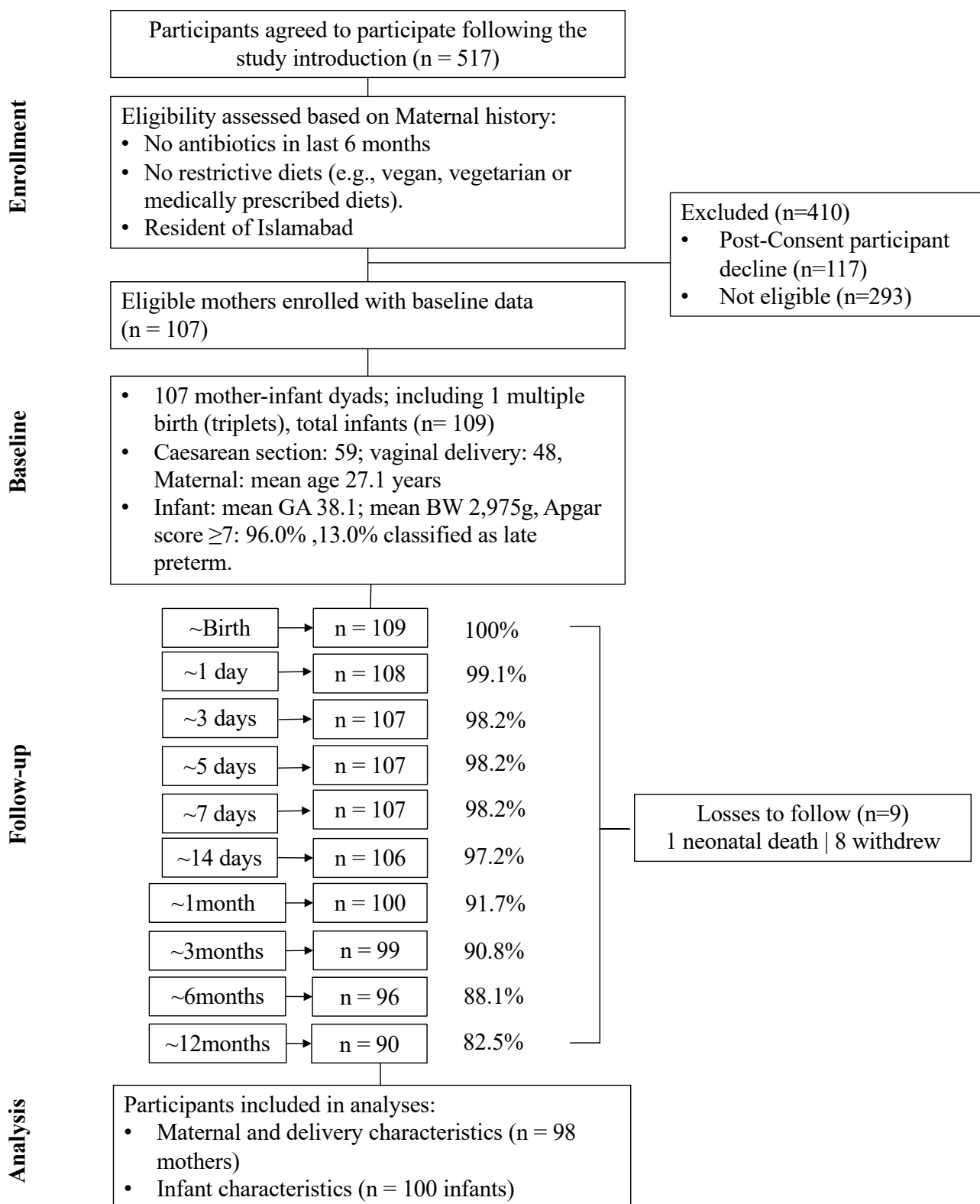


Figure 2 Participant flow diagram. A total of 107 eligible mother–infant dyads were enrolled. One pregnancy resulted in triplets, yielding 109 infants. Maternal and delivery characteristics are reported per mother (n=107), whereas infant follow-up is reported per infant (n=109). Following exclusion of 9 infants during follow-up (1 neonatal death and 8 withdrawals), 98 mothers and 100 infants were included in the baseline data analysis. GA, gestational age; BW, birth weight.

Infant Gut Bacterial Study Pakistan Cohort (INBUGS-P)

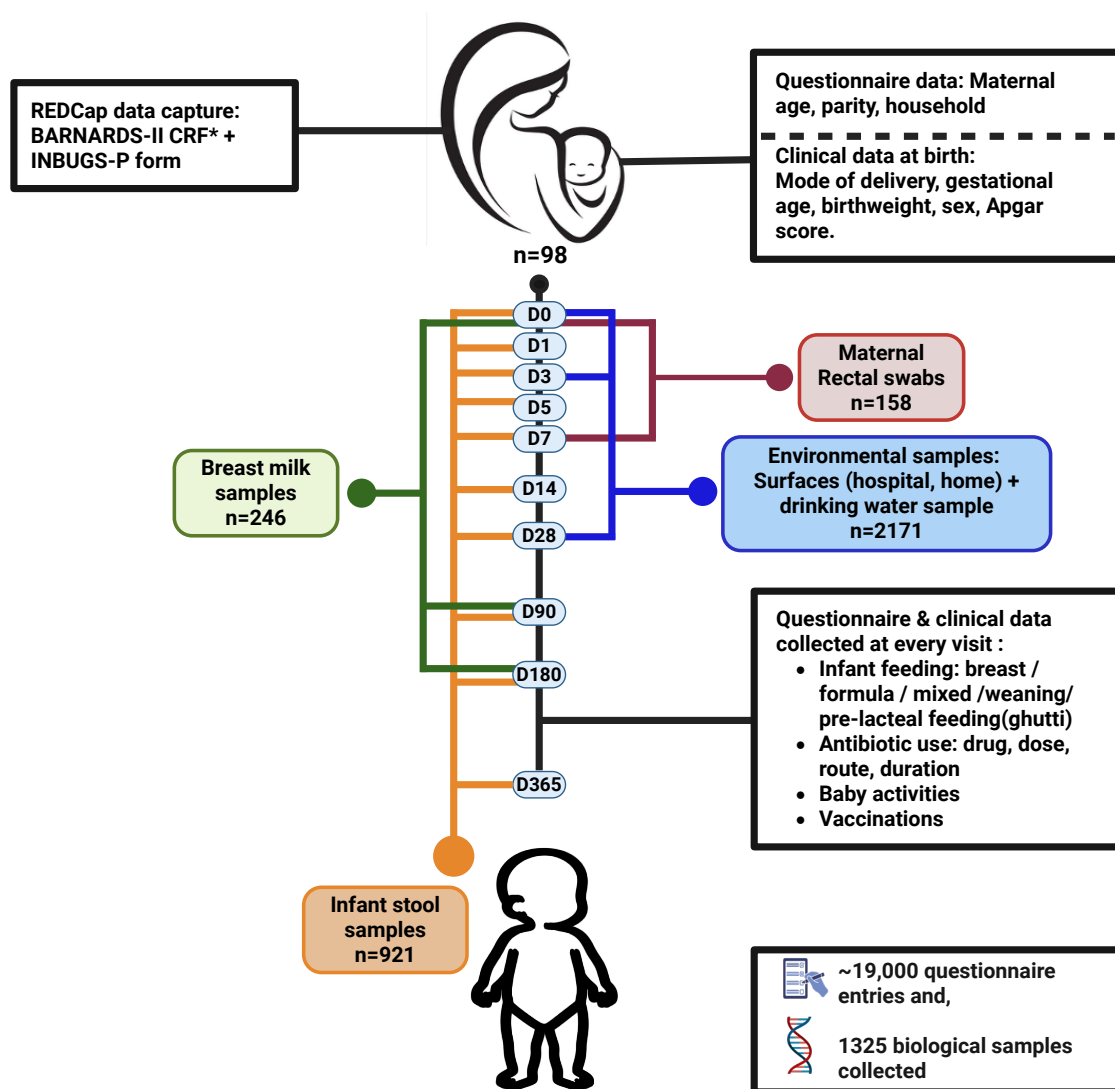


Figure 3 Infographic showing timing and types of biological and environmental samples and questionnaire data collected in the INBUGS-P cohort during the first year of life. The central vertical axis represents infant age from birth to 12 months (days 0–365). Coloured lines indicate different sample types in relation to scheduled follow-up visits. The summary panel shows the questionnaire and clinical data and the total number of questionnaire entries, biological samples and environmental samples collected during the study period. * BARNARDS-II, Burden of Antibiotic Resistance in Neonates from Developing Societies phase 2; CRF, case report form; REDCap, Research Electronic Data Capture.

practice. This included *ghutti*, a culturally prevalent practice in South Asia whereby dates or honey (varying among communities) is finger-fed to the infant in the first hours or days of birth.^{34 35} Such pre-lacteal exposures were recorded given their potential to influence early gut microbial colonisation.

Clinical data

Infant clinical variables at birth included mode of delivery, gestational age, birth weight, sex, APGAR score at 5 min and resuscitation requirements. Postpartum maternal antibiotic prescriptions and infant antibiotic exposures, including drug name, dose, route, frequency and duration, were also recorded.

Biological sample collection

Biological samples were collected from mothers, infants and hospital and household environments at predefined timepoints: day of delivery (D0), days 0, 1, 3, 5, 7, 14, 28, 90, 180 and 365, capturing key developmental stages from the immediate postnatal period through to post-weaning infancy (figure 3).

Infant stool

Ten stool samples were collected from each infant at time points days 1, 3, 5, 7, 14 and 28 and months 3, 6 and 12. Samples were collected at the bedside during the post-natal hospital stay where feasible and subsequently at home during scheduled follow-up visits. Stool consistency was recorded at each time point using predefined study-specific categories (hard, formed, loose or watery).

Maternal samples

Two rectal swabs were collected from each mother using Amies charcoal transport swabs: the first within 24 hours of delivery and the second on day 7, capturing the maternal microbiome at two distinct time points following childbirth. Breast milk samples were collected by hand expression into sterile tubes on days 0, 90 and 180 following hand washing prior to collection.

Environmental samples

Environmental surface swabs were collected at days 0, 3 and 28. At birth (day 0) and during the immediate postnatal hospital stay, hospital surfaces were sampled, including hospital bed rails and room or ward door handles. At days 3 and 28, environmental sampling was conducted in the home environment, targeting high-contact surfaces with established relevance to microbial transmission, including infant bedding, feeding areas, household door handles and caregivers' mobile phones. Drinking water samples were additionally collected from household water sources on days 3 and 28.

Sample handling and processing

All samples were transported on ice to the laboratory within 4 hours of collection. Swab samples were stored at 4°C, while stool, breastmilk and water samples were stored at -80°C until further processing. Microbial

DNA from infant stool samples was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN), following local protocol optimisation.³⁶ Extracted DNA was shipped to the University of Oxford in batches throughout the study, in line with the longitudinal recruitment schedule and sequencing workflow. Shotgun metagenomic sequencing was performed using the Oxford Nanopore Technologies GridION and PromethION 2 Solo platform with the rapid barcoding kit and R10.4.1 chemistry.

Sample size rationale

Formal sample size calculation was not performed, as no published longitudinal infant microbiome and resistome data from Pakistan were available to inform sample size estimation. An initial recruitment target of 60 mother–infant dyads was specified based on recruitment feasibility within a defined timeframe. Recruitment exceeded this target, and a total of 107 mother–infant dyads were enrolled, allowing for anticipated loss to follow-up over the 12-month follow-up period and increasing the available data for longitudinal analyses.

The repeated measures design, with 10 stool samples collected per infant, enhances statistical efficiency by enabling longitudinal mixed-effects modelling of microbiome and resistome trajectories within individuals over time, though the modest cohort size limits the power of subgroup analyses across key exposure categories.^{37 38} With only 19 infants receiving antibiotics during follow-up, analyses of antibiotic-associated microbiome perturbation are particularly limited in statistical power and would be considered hypothesis-generating rather than confirmatory. The cohort is therefore primarily descriptive and exploratory in scope, intended to generate foundational data on microbiome colonisation patterns, resistome dynamics and the influence of key early-life exposures to inform future hypothesis-driven studies in this setting.

Patient and public involvement

Patients and the public were not directly involved in the study design, conduct or reporting of this research. However, a pilot study was conducted prior to full cohort recruitment to assess the feasibility of recruitment procedures, the acceptability of longitudinal biological and environmental sample collection, home visits and the appropriateness of participant incentives. Findings from the pilot study informed recruitment strategies and study procedures. Study findings will be shared with participating families and local healthcare providers in accessible formats.

Findings to date

Maternal and household characteristics

Baseline maternal and household characteristics of the 98 mother–infant dyads are summarised in table 1. Maternal age ranged from 18 to 39 years (mean 27.1 years, SD 4.9). Nationally, the median age at first birth is 22.8 years.³⁹ Parity varied across the cohort, with a median of 2 previous pregnancies (IQR 1–3). Overall, 21.0% of women were

Table 1 Baseline characteristics

Characteristic	Value
Maternal sociodemographic characteristics (n=98 mothers)	
Age (years), mean (SD), range	27.2 (4.9); 18–39
18–24	30 (30.6%)
25–34	61 (62.2%)
≥35	7 (7.1%)
Parity (prior pregnancies), median (IQR)	2 (1–3)
Nulliparous (0)	21 (21.4%)
1–2	42 (42.9%)
≥3	34 (34.7%)
Household size, mean (SD); range	6.9 (3.4); 2–17
Children <14 years in the household, mean (SD)	2.5 (2.0)
Monthly household income (PKR), median (IQR)	30 000 (22 750–40 000)
Delivery characteristics (n=98 mothers)	
Vaginal delivery	44 (44.9%)
Caesarean section	54 (55.1%)
Premature rupture of membranes (PROM)	17 (17.3%)
Rupture-to-birth interval>18 hours	9 (52.9% of PROM)
Rupture-to-birth interval>24 hours	4 (23.5% of PROM)
Multiple birth (one triplet event, three infants)	one mother (three infants)
Length of hospital stay (days), median (IQR)	1 (1–2)
Infant characteristics (n=100 infants)	
Female	52 (52.0%)
Male	48 (48.0%)
Gestational age (weeks), mean (SD); range	38.1 (1.6); 34–41
Late preterm (34–36 weeks)	13 (13.0%)
Term (≥37 weeks)	87 (87.0%)
Birth weight (g), mean (SD); median (IQR)	2975 (479); 3000 (2600–3300)
Low birth weight (<2500 g)	10 (10.0%)
Normal birth weight (2500–3999 g)	90 (90.0%)
Apgar score at 5 min, mean (SD)	8.3 (0.8)
Environmental and cultural exposures	
Smoking in the primary household	19 (19.4%)
Ghutti given after birth	32 (32%)

i. Denominators: delivery and maternal characteristics are reported per mother (n=98); infant characteristics per infant (n=100).
 ii. One multiple birth event occurred (triplet delivery); the triplet mother contributes one delivery record and three neonatal records.
 iii. Two records had negative length-of-stay values (data entry errors) and were excluded from LOS calculation (valid n=96).
 iv. Ghutti: traditional South Asian practice of administering honey/date to infants shortly after birth.
 v. Apgar score (assessing Appearance, Pulse, Grimace, Activity and Respiration) at 5 minutes after birth.
 PKR, Pakistani Rupee; PROM, premature rupture of membranes.

nulliparous, 42.9% had one to two previous pregnancies and 34.7% had three or more previous pregnancies.

Households were relatively large, with a mean size of seven members (SD 3.4; range 2–17). The mean number of children under 14 years residing in the household was 2.5 (SD 2.0). The mean reported monthly household income was PKR 32 750 (median PKR 30 000; IQR

21 500–40 000). Household smoking was reported in 18.8% of households.

Infant and birth characteristics

Delivery and infant characteristics are summarised in table 1. Caesarean section accounted for 55.1% (54/98) of deliveries, while 44.9% (44/98) were vaginal deliveries. PROM occurred in 17 mothers (17.0%). Median hospital stay was 1 day (IQR 1–2). One multiple birth event occurred: a set of triplets delivered by planned C-section at 34 weeks.

All 100 infants were born alive with no recorded birth injuries. Mean gestational age was 38.1 weeks (SD 1.6), with 13.0% classified as late preterm. Mean birth weight was 2975 g (SD 479), similar to previously published birth-weight data from Pakistan.^{40 41} Low birth weight (<2500 g) was observed in 10.0% of neonates. APGAR scores were reassuring in 91.0% (score ≥8; mean 8.3, SD 0.8), and sex distribution was near-equal (52.0% female, 48.0% male).

Infant feeding and cultural practices

Breastfeeding was the predominant feeding mode during the first year of life. However, breastfeeding was commonly supplemented with formula feeding, and only 24% (24/100) of infants remained exclusively breastfed from birth to 6 months. This is lower than the national estimate reported for Pakistan⁴² and below the WHO recommendation of exclusive breastfeeding for the first 6 months of life. Introduction of complementary food before 6 months was observed in 16 of 100 infants, and 11 of 100 infants received water before 6 months of age. The traditional practice of administering *ghutti* was reported in 32 of 100 infants (figure 4).

Maternal and infant antibiotic exposure

Antibiotics were prescribed at hospital discharge to all mothers, regardless of mode of delivery (table 2). All prescriptions consisted of a dual oral regimen comprising a nitroimidazole and a first, second or third-generation cephalosporin to cover gram-positive, gram-negative and anaerobic bacteria (figure 5A). These antibiotics were prescribed prophylactically as part of routine clinical practice in this setting.⁴³

Among n=100 infants included in the analysis, 19 (19%) received at least one antibiotic course during follow-up, whereas 81 (81%) had no recorded antibiotic exposure. Antibiotic exposure occurred predominantly during the neonatal period (11 out of 19 infants). The most common regimen during this period was amikacin plus ceftazidime, consistent with empirical treatment for suspected neonatal sepsis in this setting.⁴⁴ Other regimens included ampicillin plus cloxacillin (n=1), metronidazole plus cefixime (n=1) and metronidazole alone (n=2). Two infants experienced repeated antibiotic exposures and received more than one regimen (figure 5B).

Follow-up and sampling

Follow-up was well maintained, particularly during the neonatal period (n=100/109, 91.7%), with gradual attrition to 90/109 (82.5%) at 12 months. Repeated sampling



Figure 4 Sunburst chart showing longitudinal patterns of infant feeding practices and antibiotic exposure. The inner ring represents follow-up time points (days 0, 1, 3, 5, 7, 14, 28, 90, 180 and 365). The middle ring indicates antibiotic exposure status at each time point (eg, use vs no use). The outer ring shows the distribution of feeding practices stratified by antibiotic exposure. Segment sizes are proportional to the number of individuals in each category.

across predefined timepoints generated a rich longitudinal dataset of biological and environmental specimens.

Infant stool samples were the primary biological samples and were obtained at 921 of 1000 scheduled timepoints (91.2%), indicating high capture across visits. Maternal rectal swabs were scheduled at two timepoints, with 158 samples collected from 98 mothers; 52 mothers provided samples at both visits, while 46 contributed a single sample, with highest completeness at birth.

Breast milk samples were scheduled at three timepoints, with 74 mothers (75.5%) contributing samples at all visits

Table 2 Delivery mode of enrolled mothers	
Delivery mode	Number of deliveries
Planned caesarean section	31
Emergency caesarean section	23
Forceps-assisted vaginal delivery	31
Spontaneous vaginal delivery	9
Induced vaginal delivery (drugs)	4

and 24 (24.5%) providing a single sample, yielding 246 samples in total.

Environmental sampling was conducted at three predefined timepoints across 98 participants, generating 2171 samples from household, hospital and water sources. The most frequently sampled surfaces included household door handles (n=444), infant bedding (n=435), sink and tap surfaces used for infant washing (n=391), caregivers' mobile phones (n=348) and drinking water sources (n=323). Hospital surface samples, including bed rails (n=101) and ward door handles (n=95), were primarily collected at birth and during the immediate postnatal hospital stay.

These findings demonstrate strong participant engagement and the feasibility of longitudinal mother–infant microbiome research with repeated biological and environmental sampling in this setting.

Strengths and limitations

Strengths

To our knowledge, INBUGS-P is the first prospective longitudinal birth cohort from Pakistan designed to

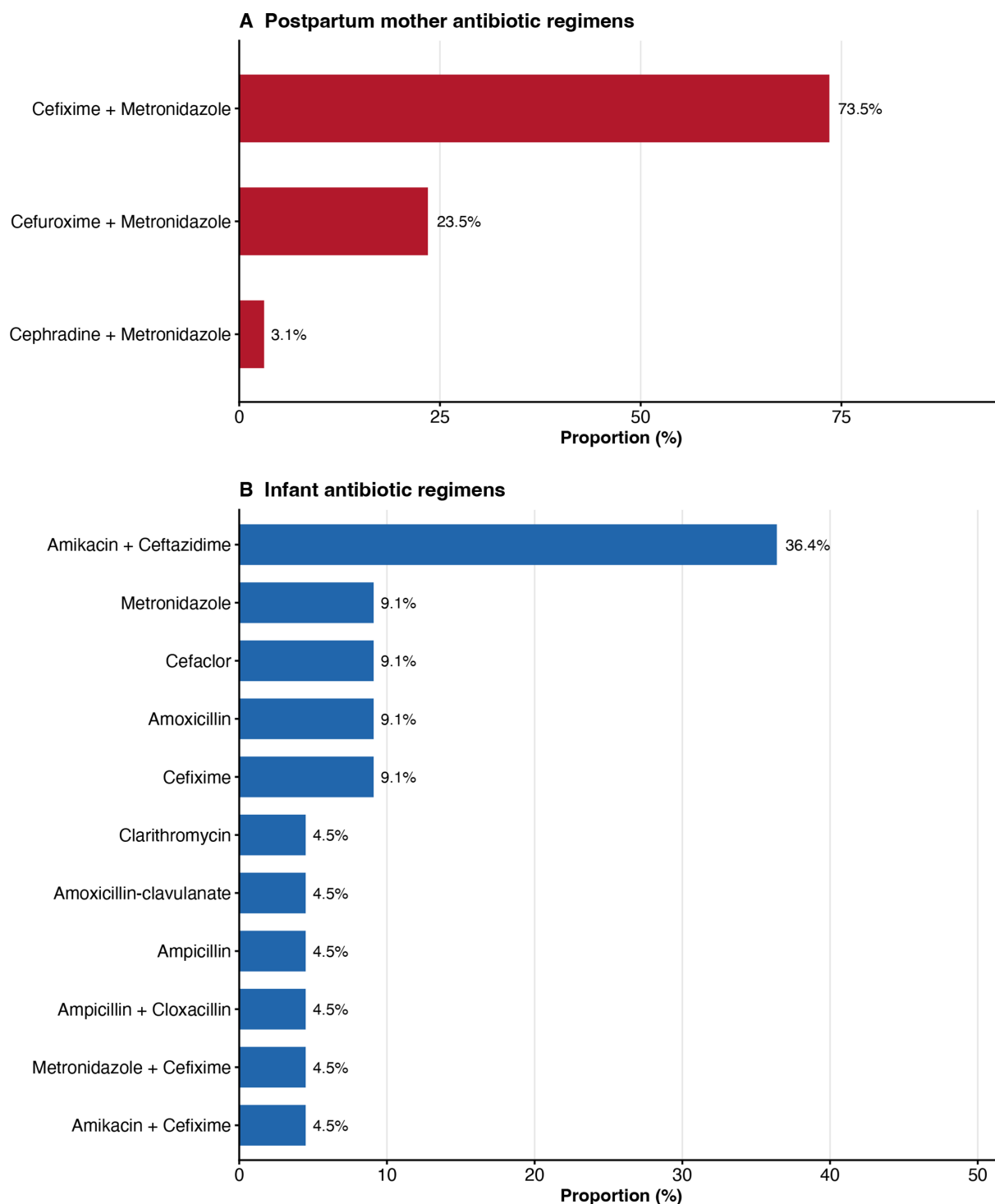


Figure 5 Maternal and infant antibiotic regimens. (A) Distribution of postpartum antibiotic regimens prescribed to mothers at hospital discharge (n=98 mothers). (B) Distribution of antibiotic regimens prescribed to infants during the first year of life. Percentages represent the proportion of antibiotic regimens prescribed to 19 infants during follow-up.

characterise early-life gut microbiome colonisation and resistome dynamics using long-read shotgun metagenomic sequencing. This approach enables species-level taxonomic resolution of microbial communities alongside characterisation of plasmid-mediated ARGs, including ESBLs and carbapenemase genes previously reported among neonates in this setting.

The study incorporates concurrent sampling of infant stool, maternal rectal swabs, breast milk and household environmental swabs from the same mother–infant dyads. These biological samples are complemented by detailed longitudinal metadata on antibiotic exposure, infant feeding practices, culturally specific exposures such as *ghutti* administration, socioeconomic status and clinical outcomes, enabling

investigation of potential microbiome and resistome transmission pathways.

The cohort was established in an LMIC setting where environmental exposures, household crowding and antibiotic use patterns differ substantially from those in high-income settings that dominate current microbiome research. The availability of detailed household demographic and socioeconomic data further allows exploration of environmental and social determinants of infant microbiome and resistome development.

The study is supported by a multidisciplinary collaboration between the University of Oxford, Quaid-i-Azam University and the PIMS hospital, with support from the Ineos Oxford Institute for Antimicrobial Research. This partnership facilitated high participant retention and repeated longitudinal sampling through home visits during the first year of life. Laboratory workflows were shared across sites, with DNA extraction performed in Pakistan prior to shipment of samples to Oxford for sequencing.

Limitations

Follow-up is currently limited to the first 12 months of life, restricting characterisation of microbiome trajectories through the second year when the gut microbiome remains dynamic and the persistence of ARGs into later childhood cannot be assessed. As recruitment was conducted at a single tertiary care hospital in urban Islamabad, findings may not be generalisable to infants born in rural areas of Pakistan or smaller district-level facilities where healthcare access, antibiotic prescribing practices and environmental exposures differ substantially. The high caesarean section rate of 55% reflects a recruitment bias inherent to hospital-based enrolment, whereby mothers delivering by caesarean section had longer postnatal hospital stays, providing greater opportunity for recruitment. In addition, postpartum antibiotic prescribing was routine at the study hospital, although routine postpartum prophylaxis is not recommended in national guidelines.⁴⁵ Several factors may have influenced local prescribing practices. A high proportion of vaginal deliveries in this cohort were forceps-assisted (table 2), which may have influenced prescribing decisions because of the perceived risk of postpartum infection. However, information on episiotomy, perineal tears, wound complications and other factors, such as local infection risk, hygiene conditions, maternal nutritional status and anaemia, were not collected in the present study; therefore, the reasons for routine postpartum antibiotic prescribing cannot be determined from our data. Home-based stool sample collection relied on parental compliance without direct researcher oversight, introducing potential variability in sample timing relative to defecation and sample quality prior to transport that cannot be fully quantified. In addition, stool consistency was recorded using predefined study-specific categories rather than a validated infant stool consistency scale. Finally, although matched infant, maternal and environmental samples enable investigation of potential transmission sources, finding the same resistance gene or organism across sample types indicates shared exposure but

does not confirm direct transmission between mother and infant or from a specific environmental source.

Future plans

Following completion of shotgun metagenomic sequencing of infant stool samples, planned analyses will examine longitudinal trajectories of gut microbiome composition and ARG acquisition from birth to 12 months of age. Microbiome diversity, taxonomic composition and resistome profiles will be evaluated in relation to key early-life exposures, including mode of delivery, breastfeeding practices, antibiotic exposure, traditional newborn practices (including *ghutti* administration) and household environment. Longitudinal mixed-effects models will be used to account for repeated sampling within individuals and to assess the temporal stability and persistence of ARG carriage.

Building on findings from infant stool analyses, the next phase of INBUGS-P will extend taxonomic and resistome profiling to maternal rectal and household environmental samples to investigate shared microbial and ARG reservoirs and potential transmission pathways within mother–infant dyads and domestic environments.

Aliquots (20 µL) of extracted DNA from stool samples have been preserved to support future hybrid sequencing approaches integrating long- and short-read technologies. This will complement existing long-read metagenomic analyses by enhancing strain-level resolution and improving the accuracy of ARG identification, including variant-level characterisation.

Comparative analyses are planned with the INBUGS-N cohort in Kano, Nigeria³² to assess the generalisability of findings across geographically distinct LMIC settings from a unified study design, sample collection and analyses. These cohorts include mother–infant pairs with comparable longitudinal demographic, socioeconomic and dietary data, enabling cross-regional comparisons of early-life gut microbiome and resistome development.

Subject to funding availability, follow-up may be extended to 24 months to characterise longer-term microbiome maturation and the persistence of resistance genes beyond infancy.

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Collaborators We welcome collaboration with researchers interested in maternal–infant microbiome dynamics, antimicrobial resistance and early-life exposures in low-resource settings. Interested collaborators should contact the Ineos Oxford Institute (<https://www.ineosoxford.ox.ac.uk/contact-us>), outlining their research objectives and proposed use of the data. Access will be granted for non-commercial academic purposes and will be subject to a data-sharing agreement. Restrictions may apply for sensitive variables or where limited by participant consent.

Contributors RT, KT, BH, TRW, RZ and KS led protocol development, and RT and RZ led the field implementation. RZ acts as the guarantor. RT, AS and RZ coordinated participant recruitment at PIMS, household follow-up, questionnaire administration and environmental sampling. JO and EOO developed the REDCap database and are responsible for data management. AKL developed the bioinformatics approach and contributed to the analysis. SA acts as the responsible study doctor. RZ, AS and SA provided site oversight, clinical governance and liaison with hospital services. KT,

RZ, TRW and KS supervised laboratory workflows and the overall conduct of the study. RT drafted the manuscript, and all authors critically revised the manuscript and approved the final version. TRW and KS contributed to funding acquisition. KT, RZ, TRW and KS contributed to study supervision. ChatGPT (GP-5.3 model, open AI) has been used minimally to assist with language editing and refinement. All outputs were reviewed, and the authors take full responsibility for the content.

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