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BMJ Open

Neonatal acquisition of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the community of a low-income country (NeoLIC): protocol for a household cohort study in Moramanga, Madagascar

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ABSTRACT

Introduction Data regarding the acquisition of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) in neonates at the community level are scarce in low-income and middle-income countries (LMICs), where the burden of neonatal sepsis is high.

Our study aims at identifying and quantifying the role of the different routes of ESBL-PE transmission for neonates, which are still undefined in the community in LMICs.

Methods and analysis In a semirural community in Madagascar, 60 mothers and their neonates will be recruited at delivery, during which a maternal stool sample and meconium of the newborn will be collected. Home visits will be planned the day of the delivery and next at days 3, 7, 14, 21 and 28. Stool samples from the newborn, the mother and every other household member will be collected at each visit, as well as samples from the environment in contact with the newborn (food, surfaces and objects). Sociodemographic data and factors which might drive ESBL-PE acquisition will also be collected.

We will analyse the isolated ESBL-PE using DNA sequencing methods to characterise clones, resistance genes and plasmids of ESBL-PE. To analyse these data globally, we will develop novel analytical approaches combining mathematical modelling and statistics. Finally, mathematical simulations will be performed to test different strategies of control of ESBL-PE transmission to neonates.

In complement, we will conduct an anthropological investigation to understand local environments and practices that would contribute to neonatal ESBL-PE acquisition. In-depth interviews with members of 16 households will be conducted and 4 mother–newborn pairs will be followed by a participants' observations methodology.

Ethics and dissemination The study was approved by the ethical committee in Madagascar and by the institutional review board of Institut Pasteur, Paris, France. Findings will be reported to participating families, collaborators and local government; presented at national and international conferences and disseminated by peer-review publications.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Collecting new longitudinal data among neonates at the community level in Madagascar; where the burden of neonatal infection is huge and using of up-to-date microbiological culture methods and integrating whole genome sequence typing and plasmid analyses.
- ⇒ Formalising new hypothesis-driven mathematical models to describe the dynamic processes occurring in the neonates.
- ⇒ Integrating an anthropological investigation to understand local environments and practices, which should help to identify the most acceptable interventions to prevent extended-spectrum beta-lactamase-producing Enterobacteriaceae acquisition and infection in neonates.
- ⇒ Due to financial constraints, we can only follow-up households up to the first month of life of newborns. However, this period is the most vulnerable in terms of morbidity and mortality.
- ⇒ We will not be able to conduct plasmid tracking per se as long-read sequencing will not be performed.

BACKGROUND

Globally, 2.4 million neonatal (<28 days) deaths occurred in 2019, and almost half of them occurred in Sub-Saharan Africa.[1] Among the leading causes of global neonatal death are severe bacterial infections.[2] The burden is particularly important in low-income and middle-income countries (LMICs): in 2012,

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it has been estimated that 6.9 million cases of neonatal possible severe bacterial infection occurred in neonates.[3]

In LMICs, neonatal bacterial infections are mainly caused by Enterobacteriaceae, more specifically *Escherichia coli* and *Klebsiella pneumoniae*.[4] One important driver of unfavourable outcomes in infections caused by these bacteria is their multidrug resistance, which challenges appropriate therapy.[5 6] Of particular concern are the extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE), which are resistant to most penicillin and especially cephalosporin (3rd–4th generations) antibiotic classes.[7] Carbapenems remain one of the last resort antibiotics to treat neonatal ESBL-PE infections. Unfortunately, carbapenem antibiotics are neither available nor affordable for populations living in LMICs.[6 8]

First reported in hospital settings, ESBL-PE emerged in the community in the early 2000s.[9] However, most data come from hospitals in LMICs whereas these settings combine a number of risk factors for the dissemination of ESBL-PE in the community, including suboptimal hygiene conditions, household crowding, poorly controlled antibiotic consumption, and insufficient infections' prevention and control. Data from the community are scarce and available ones show high ESBL-PE carriage prevalence. In Madagascar, the estimated prevalence of ESBL-PE carriage in pregnant women was 18.5% (95% CI, 14.5% to 22.6%) (compared with <5% in Europe). These findings demonstrate the need to work at the community level where the ESBL-PE prevalence is significant.[10 11]

In addition, the incidence of culture-confirmed severe neonatal infections in the community of Madagascar, one of the poorest countries in the world, was estimated to be high: 15.2 cases/1000 live births (95% CI, 10.6 to 21.8) and over a quarter of them was caused by an ESBL-PE.[12] These findings highlight the high burden of neonatal infections and the need to work at the community level.

They also underscore the importance to better understand ESBL-PE acquisition in neonates, which is the first step of infection. Indeed, the incidence rate of first ESBL-PE acquisition was 10.4 cases/1000 newborn-days (95% CI: 8.0 to 13.4) at the community level in Madagascar. Importantly, half of these first ESBL-PE acquisitions occurred during the first week of life. However, data regarding the acquisition of ESBL-PE during the neonatal period are scarce in the community. The few studies focusing on ESBL-PE acquisition in neonates were conducted in neonatal care units, where neonates are often premature and exposed to specific factors increasing the risk of ESBL-PE acquisition (eg, invasive procedures, contact with contaminated equipment or medical staff, high antibiotic consumption).[8 13 14] In the community, the newborn is exposed at birth and during the first weeks of life to several possible sources of ESBL-PE acquisition, for example, maternal, healthcare facilities and outside hospitals: parents, other relatives, food and environment. However, these sources have not yet been investigated in a community setting.

The criterion of cleanliness and the appreciation of dirt can change according to society, social class and gender.[15] This can influence the way people interact with their environment and consequently drive neonate contact pattern with its environment. Also, newborn feeding practices may differ among populations and cultures. Depending on local beliefs, neonates might be supplemented with formula, cow/goat milk, sugar water, teas or solid foods (porridge) early on.[16] Understanding of cultural beliefs and local practices, which drive neonate contact patterns with its environment and feeding practices, is essential to fully decipher routes of ESBL-PE acquisition. However, this has never been investigated among neonates in Madagascar.

Because of all these gaps, the routes of ESBL-PE acquisition in neonates are not well assessed. In order to consider the best control policies, increasing our knowledge of the most relevant ESBL-PE transmission routes in neonates in the community are of the utmost importance, especially in LMICs.

We present here the study protocol as approved by the ethical committees in France and Madagascar. At the time of submission of this paper, enrolment has begun in April 2021. Data collection and laboratory analyses are currently on-going. The expected recruitment period is 15 months.

Study objectives

This multidisciplinary research project has two aims. First, we aim to identify and quantify the different routes of ESBL-PE acquisition in neonates within a community setting of Madagascar. This will include the use of cutting-edge techniques such as whole genome sequencing (WGS) to ascertain bacterial transmission and the development of a new methodological framework (based on multilevel dynamic modelling and statistical inference) to study the between-host transmission of ESBL-PE. The second aim is to identify local beliefs and practices that facilitate neonatal ESBL-PE acquisition. At the end, we will propose an acceptable intervention for the local populations to prevent ESBL-PE acquisition in neonates by considering local practices and environment.

METHODS

Study design

This will be a prospective, longitudinal, observational community-based study in Moramanga (population 17 159 and 3795 women of childbearing age), a semirural area located 110 km east of Antananarivo, the capital of Madagascar. Pregnant women will be identified during their third trimester of pregnancy at the primary healthcare centre. At delivery, the mother with her newborn and all household members will be recruited. Inclusion criteria of the mother, neonate and household member are presented in table 1.

Stool samples (5 g per sample) of the mother and newborn (meconium) will be collected at delivery.

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Table 1 Inclusion and exclusion criteria

Inclusion criteria for mother	Exclusion criteria for mother
► Monofoetal pregnancy	► Non-monofoetal pregnancy
► Resident in study area with no plans to move away during the period of the study	► Plans to move away during the period of the study
► Agrees to participate in study and provided written consent. If minor, has assented and guardian or parent provided consent	► Does not consent ► Mother and neonate returned home before stool sample collection of the household members took place
Inclusion criteria for neonate	Exclusion criteria for neonate
► Live birth ► Parents or guardians reside in study area with no plans to move away during the period of the study ► Parents or guardians agree that the neonate participate to the study and provided written consent	► Stillbirth ► Parents or guardians reside in study area with plans to move away during the period of the study ► Parents or guardians does not consent ► Mother and neonate returned home before stool sample collection of the household members took place
Inclusion criteria for member of the household	Exclusion criteria for member of the household
► Lives in the same household as the mother and neonate	► Does not live in the same household as the mother and neonate
► Resides a minimum of 4 nights per week in the household ► Agrees to participate in study and provided written consent. If minor, has assented and guardian or parent provided consent	► Does not reside a minimum of 4 nights per week in the household ► Does not consent ► Mother and neonate returned home before stool sample collection of the household members took place
	► The household is excluded if a member of the household does not agree to participate or does not consent

Importantly, before the mother and the newborn go back home after leaving the maternity, the first sampling of every permanent household member (defined as an individual sleeping in the home≥4 nights/week) will be performed to have a baseline of ESBL-PE colonisation pressure in the household. Then, home visits by field investigators will be planned at days 3, 7, 14, 21 and 28 (figure 1: study design of the NeoLIC project). At each visit, stool samples will be collected from the newborn, mother and every other permanent household member. Also, sampling of newborn's food will be collected, mainly breast milk (5–7 mL), water or other liquids (15–25 mL) given to the newborn. Surfaces and objects in contact with the newborns (eg, pacifier, toys) will be swabbed at each visit. The area covered by these swabs will be 25cm^2, when possible; if not, the maximum area will be swabbed and the area of the sampled surface will be recorded to quantify the number of colonies per cm^2.

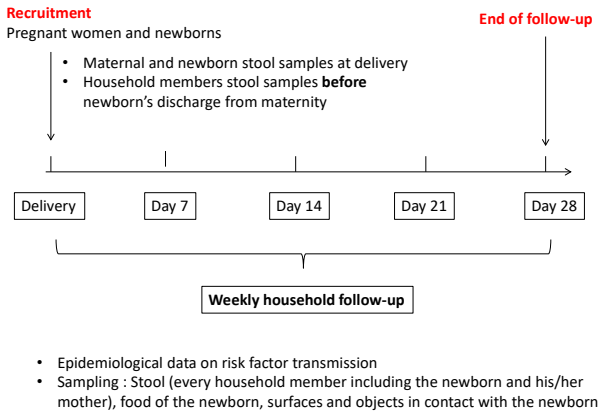

Figure 1 Study design of the NeoLIC project.

In complement, data on the gestation and the mode of delivery will be collected. Sociodemographic characteristics, medical history and risk factors for carriage and transmission (such as diet and hygiene habits, contact with animals), hospitalisation, consumption of antibiotics in the previous days, will also be documented for each participant.

Sample size

In total, a minimum of 60 households will be recruited. On the basis of national data and previous results, seven people on average are expected per household.[17] Among the 60 identified households, a total of 420 individuals are therefore expected to be followed up, leading to an estimate of 5040 collected samples. Based on our previous experiences, we estimated an ESBL growth of 30%. Thus, among the 5040 estimated samples to be collected, we expect to isolate a maximum of 1512 ESBLs. The human resources required, the number of households to be included at a monthly basis (four households per month) and the duration of the inclusion period (15 months) were determined so as not to overload both the teams working in the field and in the laboratory.

The enrolment of ~420 subjects clustered within 60 households would allow detecting a minimum OR of 1.17 for factors associated with colonisation with 80% power and α=0.05.

Also, as the aim of this study is to assess transmission routes of ESBL-PE acquisitions in newborns, routes will be assessed using mathematical dynamic modelling.

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The statistical power of this approach relies on having a sufficient number of longitudinal time points and events (acquisition days and no acquisition days, colonisation periods of all sources). Based on our previous studies, we expect 40 acquisition and 260 non-acquisition events, which is appropriate to study pathogen transmission routes for the dominant strains.[18 19]

Bacteriology

All samples will be placed in a labelled, sterile, leak-proof and preservative-free container. They will be transported in a portable cooler with ice packs within 8–12 hours to the Institut Pasteur in Madagascar (IPM), Antananarivo, for analyses. Temperature during transport will be monitored.

On arrival at the IPM, temperature will be recorded in a logbook and stool and surface samples will be processed in the laboratory of experimental bacteriology unit at the IPM. The food samples will be processed by the laboratory of food and environmental hygiene at the IPM within 24 hours after collection or kept frozen at −20°C until analyses.

Samples will be first pre-enriched according to Jazmati *et al*,[20] and subsequently inoculated on CHROMagar ESBL (CHROMagar, Paris, France) and incubated aerobically for 24 hours at 36.5°C±2°C. Representative colony morphotypes will be identified by mass spectrometry matrix assisted laser deionization time of flight (MALDI–TOF MS; Biotyper V.3.3, Bruker Daltonics, Champs-sur-Marne, France). The colonies will be selected based on their colour and morphological appearance such as size, surface and edge. Based on our previous experiences, we expect that in stool the majority of the colonies will belong to the Enterobacteriaceae and will be pink (*E. coli*) or metallic blue (*Klebsiella* spp) and that mixed cultures containing two or more morphologically distinct colonies will be frequent. Colonies from each colour present will be selected with the exception of blue colonies of which a maximum of three colonies will be selected each showing a different appearance and with a preference for the metallic blue colonies, if applicable. Cultures showing mixtures of colonies will be subcultured until pure isolated colonies are obtained for further processing. Only single and pure bacterial colonies will be processed for identification and if *Enterobacteriaceae* also for antibiotic susceptibility testing and DNA extraction for WGS.

Antimicrobial susceptibility testing will be performed on each *Enterobacteriaceae* isolate. Amoxicillin, ticarcillin, ticarcillin–clavulanic acid, cephalothin, amoxicillin–clavulanic acid, aztreonam, cefotaxime, ceftazidime, cefepime, imipenem, ertapenem, cefoxitin, cefuroxime, gentamicin, nalidixic acid and ciprofloxacin will be tested. The presence of ESBL-PE in isolates will be confirmed by the double-disk synergy test by placing the disk of cefotaxime (30 µg), ceftazidime (30 µg) and combination of amoxicillin/clavulanic acid (20 µg/10 µg) on a lawn culture of bacteria on Muller-Hinton agar plate, with a 20 mm distance between each disk from centre to centre. The plates will be incubated aerobically for 24 hours at 36.5°C±2°C. Isolates for which we will observe an enhanced inhibition zone toward amoxicillin–clavulanic acid and cefotaxime or ceftazidime will be considered as possible ESBL producers.[21] *E. coli* American Tissue Collection Culture 25 922 will be used as internal quality control. The phenotype interpretation will be done according to the Comité de l'Antibiogramme de la Societe Francaise de Microbiologie (CASFM)/European Committee on Antimicrobial Susceptibility Testing(EUCAST) guidelines (http://www.sfm-microbiologie.org).[21]

Aliquots of stools and isolates will be stored at −80°C for future investigations.

Whole genome sequencing

All ESBL isolates will be selected for WGS. DNA will be extracted from each ESBL-PE isolate using QIAamp 96 DNA QIAcube extraction kits on an automated DNA QIAcube extraction platform (Qiagen, Germany) at IPM.

Whole genome sequencing (coverage depth>50×) will be performed at the Plateforme de Microbiologie Mutualisée, Institut Pasteur, Paris. Libraries will be constructed using the Nextera XT DNA library preparation kit (Illumina, San Diego, California, USA) and sequenced on a NextSeq 500 instrument using a 2×150 bp paired-end (PE) protocol. Taxonomic assignment will be performed in silico from the PE reads using Kraken 2 for assessing the initial bacteriology laboratory assignments in order to determine putative contaminations.[22] Genome sequences will be assembled and annotated using the mini-workflow fq2dna (https://research.pasteur.fr/en/tool/fq2dna). Briefly, after preprocessing the PE reads (ie, deduplication, trimming, clipping, putative contaminating human read removal, sequencing error correction, digital normalisation), fq2dna infers two de novo assemblies (ie, with and without PE read merging) using SPAdes and selects the less fragmented one.[23] Next, fq2dna filters out troublesome contigs (too short and/or significantly under covered by the PE reads). Finally, the assembled scaffolds are annotated using Prokka and classified as chromosome/plasmid using Platon.[24 25] In order to characterise the plasmid content of each sequenced and assembled isolate, the identified plasmid scaffolds will be next typed using PlasmidFinder and the last release of the associated Enterobacteriaceae replicon databank (https://bitbucket.org/genomicepidemiology/plasmidfinder_db/src/master).[26] This plasmid sequence typing step will therefore allow studying the plasmid transmission.

In complement, the determination of the acquired resistance gene content of each sequenced and assembled genome will be performed using ResFinder and the last release of the associated reference sequence database (https://bitbucket.org/genomicepidemiology/resfinder_db/overview).[27]

Thanks to the scaffold classification using Platon, each determined resistance gene will be assessed as carried on the chromosome or plasmid(s), therefore allowing

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the tracking of possible resistance gene transfers from plasmid to chromosome.

Bacterial clone tracking

Multiple de novo assemblies of the same bacterial clones will be available through the longitudinal follow-up, therefore opening up the possibility to measure the overall similarity between each pair of assembled genomes (eg, Basic Local Alignment Search Tool-or MinHash-based nucleotide identity measures) that belong to the same clone. As two assembled chromosomes are never fully identical (eg, substitution events, sequencing or assembling errors), such similarity values based on pairwise genome comparisons will allow intraclone similarity thresholds to be determined for each ESBL-PE strain considered during this study (for a similar approach, see e.g.[28]). Based on these intraclone thresholds determined a priori, we will be able to infer a posteriori that two isolates belong to the same clone, therefore leading to the accurate follow-up of bacterial clones within each of the different households.

As a final step, these overall bacterial clone classifications will be confirmed by different phylogenetic analyses at the genome level using JolyTree and Parsnp.[29 30] As the chromosome contig set was determined for each sequenced sample (see above), the phylogenetic classification of these chromosome sequences will be carried out for each species, therefore enabling the identification of the different sampled clones.

Anthropology procedures

The families will be enrolled for semi-structured, informal interviews and participant, direct observations to put the epidemiological, microbiological, genomic and modelling results into perspective in a more global context, particularly in both cultural and economic contexts of an LMIC such as Madagascar.

In-depth individual interviews with parents and caretakers of newborns

Among the 60 households recruited, in-depth interviews will be conducted with some members (16 mothers, 4 fathers, 4 mothering adults and 4 siblings) of 16 households. The households will be selected among two groups according to neonatal ESBL-PE status: (1) household in which the neonate has never acquired EPBL-PE during the whole follow-up and (2) household in which the neonate had at least one stool positive for ESBL-PE during the follow-up. In addition, we will also interview others potential informants, such as community health workers, traditional birth attendants, neighbourhood chief, traditional healer, local physician and nurse. Interview grids will be prepared for each category of person targeted to explore four themes: (1) history of the mother–newborn dyad, (2) feeding practices, (3) hygiene and (4) newborn's mobility and interaction with physical and social environment. This will enable us to identify daily and seasonal environmental practices regarding the child's interaction with his/her environment and caregivers through local social norms. Each interview will be conducted individually to avoid any possible influence of members of the respondent's entourage on his/her response.

The interviews will be conducted in local language (Malagasy) and recorded after participant's approval.

Participant and direct observation

Observation grid will be used to document (1) practices including care, feeding, hygiene (such as defecation practices, hand washing) and water storage and use and (2) interactions with the environment of the neonate and others family members in their daily life. We will select four families with mother–newborn pairs: two pairs for which the newborn has never been ESBL-PE colonised and two other pairs for which the newborn has been ESBL-PE colonised during the follow-up. These observations will lead us to identify activities undertaken on a daily/seasonal basis (observations will be made both outside and during the rainy season), where these activities take place, and with whom. This will offer insight into the differences between what people say and what they do on a daily basis.

Patient and public involvement

Patients were not involved in the formulation of the research question, study design or recruitment of the study. Our multidisciplinary study, including anthropological investigation, should suggest the most acceptable interventions for the local population to prevent ESBL-PE acquisition and infection in neonates taking in account practices and environment of the community.

Findings will be reported to participating families and collaborators at the end of the study.

DATA ANALYSIS

Statistical and mathematical modelling

These analyses will address the question of identifying and quantifying the role of the different routes of transmission for newborns at home, by developing a specific mathematical modelling framework. Finally, simulations to assess different strategies of control of ESBL-PE transmission to newborn in households will be performed.

Statistical analysis of newborn acquisition risk factors

Qualitative variables will be expressed as counts (percentages) and quantitative variables as mean (SD) or median (IQR) for non-normal distributed variables. Incidence rates of the first acquisition and clearance of ESBL-PE per 1000 newborn-days will be estimated. Fisher's exact test will be employed for all 2×2 contingency table tests. Kruskal-Wallis non-parametric one-way analysis of variance will be used for pairwise comparisons between sets of continuous observations and Spearman's non-parametric rank correlation for calculating correlation between two covariates. To identify factors associated with first ESBL-PE acquisition in newborns, we will use a multivariable generalised mixed effects logistic regression

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model with random effects for individual and household included to control for repeated sampling. We will consider variables potentially associated with ESBL-PE acquisition, such as sociodemographic factors, parity, newborn's characteristics, antibiotic consumption and hygiene. All factors associated with the outcome with a p value<0.20 in univariate analysis will be entered in the multivariate model. A backward selection procedure will be applied to identify factors independently and significantly associated with the outcome. Potential interactions will be tested.

A p value<0.05 will be considered significant.

This step will enable to identify risk factors associated with ESBL-PE acquisition, which will then be considered in the mathematical modelling.

Mathematical modelling of newborn acquisition

A mathematical model of transmission of ESBL-PE in a household will be built to estimate for each bacteria or resistance gene the contribution of the different routes to newborn's acquisition. The model will be structured according to the newborn individual characteristics: his/her colonisation status to the studied bacteria (colonised, non-colonised), the household characteristics (family members, newborn/mother/brother, sister…), and other risk factors selected from the statistical analysis. The following processes will be formalised: (1) within the host: multiple colonisation by species, including the possibility of gene transfer for ESBL acquisition (plasmids transmission), impact of antibiotic exposure, natural clearance; (2) between hosts: transmission through the different human routes (eg, mother, siblings, breastfeeding, food), acquisition of species or resistances, epidemiological data (known contacts); and (3) between the newborn and its environment: for example, contacts with contaminated surfaces, water, food, objects.

The force of infection, which represents the risk of acquisition to which each susceptible newborn is exposed by unit of time, will be written as a sum of different components describing the different possible routes of colonisation.

The model will be integrated in a statistical inference framework to analyse the data and estimate unknown model parameters (eg, transmission rates, impact of antibiotics). For each household, the model will be parameterised and initialised using the collected data at the individual level in each household, for example, individuals' status, antibiotic exposure, age, environmental swabs results. It will then be confronted to the collected data over time, and optimal parameters (ie, enabling the best reproduction of the longitudinal data) will be estimated. Due to the model complexity and the diversity of transmission routes assessed, a series of models of increasing complexity will be built to compare their likelihood given the data and acquisitions observed over time in the different households. The different models will then be compared based on chosen criterion, for example, the Deviance Information Criterion to select the best model in terms of its ability to reproduce the data but also discriminates models according to their complexity. That step will enable a detailed quantitative characterisation of the key biological and sociological processes at interplay in ESBL-PE transmission to newborns.

In case of missing data, the underlying mechanism for why the data are missing will be considered. If possible, sensitivity analyses incorporating worst-case and best-case scenarios will be performed for both statistical and mathematical modelling.

Evaluation of impact of interventions: simulation study

The parameterised model selected in the preceding step will be used to simulate and assess different strategies of control of ESBL-PE transmission to newborns in households. In particular, we will assess the impact (1) of modifying behaviours (patterns of human contacts), (2) of modifying drug regimens and (3) of hygiene (decontamination of objects or surfaces).

Anthropological analysis

All interview and observation notes will be transcribed and translated into French. The analysis will be carried out according to the thematic analysis method, which will consist of dividing the texts into basic units for assessment. Next, each unit will be codified and then grouped into homogeneous categories. Analysis grids will be then filled out according to the homogenous themes. This method will enable us to identify recurrences and divergences in the statements made depending on the issues addressed.

Ethics and dissemination

Written informed consent will be obtained from all participants. In case the participant is minor (<18 years old), written informed consent will be asked to a parent or guardian of the participant. Assent will be asked to study participants aged 7–17 years.

Data protection will follow both European Union and Malagasy data protection rules. Finally, all biological material (DNA) will be transferred in accordance with the international rules.

The study was approved by the ethical committee in Madagascar (N° 190 MSANP/SG/AGMED/CERBM) and by the institutional review board (n° IRB2020-010) of Institut Pasteur, Paris, France.

Findings will be reported to (1) participating families; (2) institutes and collaborators supporting the study; (3) local government to inform policy; (4) presented at local, national and international conferences; and (5) disseminated by peer-review publications.

DISCUSSION

Our study will aid in the development of strategies to prevent and reduce the neonatal acquisition of ESBL-PE in the community and improve early newborn care in Madagascar. Consequently, we will discretely contribute

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in the actions to progress towards the sustainable millennium development goal 3.2 target, which aims to reduce neonatal death to less than 12 per 1000 live births for each country by 2030.[1]

To reach this aim, it will require an innovative longitudinal data collection among neonates in Madagascar, where very few studies in the community have been conducted. Also, the spread of ESBL-PE is a complex process involving both transmission of predominant clones (person to person) and horizontal gene transfer of the responsible resistance genes between different clones and bacterial species (plasmid transmission). In Madagascar, very scarce molecular information on circulating ESBL-PE is available, with a real lack of genomic data.[8 31] This knowledge is essential to follow the transfer of beta-lactamase genes and the dissemination of resistant clones. Integration of whole genome sequence typing and plasmid analyses are therefore necessary in the source identification, and support putative transmission events and their direction.

Mathematical models are useful tools to gain insight into the dynamics of antibiotic-resistant bacteria transmission and to predict the impact of control measures. Existing models either focus on the transmission of ESBL resistance or attempt to reconstruct transmissions by building phylogenetic trees from genomic sequences. However, these approaches should not be looked at independently. A quantitative framework is still lacking to analyse in an integrative manner different levels of temporal data, from between-host interactions to microbiological and genomic analyses of ESBL-PE and epidemiological data.

Finally, local understandings of practices are essential to fully decipher routes of ESBL-PE acquisition in the context of LMICs. Indeed, intervention implementation needs to take in account local cultural practices to be widely acceptable by the population.

One strength of this study is to fill all these gaps by (1) collecting new longitudinal data among neonates in low-income countries at the community level, (2) using of up-to-date microbiological culture methods, (3) integrating whole genome sequence typing and plasmid analyses, (4) formalising hypothesis-driven models to describe the dynamic processes occurring in the newborns and (5) integrating local environment practices understanding that would contribute to ESBL-PE transmission. Another strength of our study come from highly complementary ranges of expertise to be pooled: field epidemiology, anthropology, microbiology, genomics, bioinformatics, modelling, biostatistics, medicine and public health. This multidisciplinary approach is required for the successful implementation of control strategies of ESBL-PE acquisition in newborns in Madagascar, where the burden of neonatal infection is important.

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