International Pathogen Surveillance Network: Catalytic grant fund

February 2024

Concept note template

Please complete all sections below in English. Incomplete applications will not be considered for funding. Should you have any questions on your submission, please submit them according to the instructions in the Request for Proposal (RfP). Please note: any research involving human participants and/or individual data will be subject to additional ethical review.

If your organization is ineligible for funding, e.g., because it is based in a high-income country, but wishes to pitch a relevant project idea to be matched with an organization based in a low- or middle-income country, please fill out the associated concept note with a null budget line and include suggested modalities for collaboration in the appropriate sections.

1. Applicants

1.1. Lead applicant

First/Given Name	Inácio
Last/Family Name	Mandomando
Current Position	Coordinating Researcher and Coordinator of Bacterial, Viral and Neglected Tropical Diseases Research Area
Organization	Fundação Manhiça
Address of Applicant's Organization	Rua 12, Bairro Cambeve, Vila da Manhiça, Maputo, Mozambique; CP 1929
IPSN membership? (Yes/No/In progress)	Yes
E-mail address	inacio.mandomando@manhica.net
Phone number	+25821810002

1.2. Partner organizations (if any)

Please insert additional rows as needed to list all partner organizations included in the application.

Organization Name	Organization's WHO Member State, Territory or Area	Co-applicant's Full Name	IPSN membership? (Yes/No/ In progress)	Brief description of role on project
Statens Serum Institute	Denmark	Nadia Boisen	In progress	Mentorship and training of Mozambican scientists in bacterial WGS methods, data analysis and technical support

If no partner organizations are listed above, is your organization interested in the IPSN suggesting potential collaborators?

☐ Yes

☐ No

☒ Not applicable

1.3. Technical and collaboration experience

Please outline how the lead and co-applicant's technical experience in pathogen genomic surveillance and experience working in collaboration will contribute to the success of this project (200 words max).

The lead applicant, Dr Mandomando is a leading expert with >15 years of experience in conducting diarrheal and invasive bacterial disease (IBD) research in Africa with interest in molecular epidemiology, including genomic surveillance of bacteria (*Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*) and viruses (rotavirus, SARS-CoV-2).

Dr Boisen is a leading expert on *E. coli*, analysing the pathogen interactions in the mammalian gut with focus on adhesion, and epithelial barrier integrity, proposing a pathogenesis model for the O104:H4 STEC-EAEC German outbreak strain. She has collaborated in global projects (Global Enteric Multicentric Study-GEMS), and leads the OH-HARMONY-CAP: One Health Harmonisation of Protocol for Detection of Foodborne Pathogens and AMR determinants and a BMGF-funded project on neonatal sepsis by *Klebsiella pneumoniae* and *E. coli.*

The applicants (Dr Mandomando and Dr Boisen) collaborate since 2010, characterizing pathogens at genomic level (Mandomando *et al.* 2020; Boisen *et al.* 2020). Some of their collaborations include the characterizion of Extraintestinal Pathogenic *Escherichia coli* (ExPEC) causing bacteremia in Mozambican children, with description of a new sublineage (ST131, *fimH*27) harbouring virulence factors found in enteroaggregative *E. coli* (EAEC) and ExPEC, and associated with high mortality. They are also working on the WGS of poultry *Salmonella* isolates from Mozambique.

2. Project proposal

2.1. Title: Genomic Insights into Antimicrobial Resistance: Unveiling its Impact in Southern and Central Mozambique

2.2. Significance

Please outline the challenges and/or knowledge gaps addressed by the project, the broad aims and/or proposed solutions, the geographies covered, how the project links to IPSN areas of work, and the expected impact (500 words max).

Antimicrobial resistance (AMR) has emeged as a public health threat, particularly in low and middle coutries (LMICs). The global burden of AMR, estimated 4.95 million deaths associated with bacterial AMR in 2019, of which 21% (1.05 million) occurred in sub-Saharan Africa (SSA) (Murray *et al*. 2022). In these settings, ESKAPE-E (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae [Kp]*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*) are ranked among the top pathogens associated with mortality (Sartorius *et al.* 2023). These pathogens are often characterized as multi- “MDR”, extensively- “XDR” and pandrug “PDR” resistant, posing a significant threat to the public health, as they are commonly related with treatment failure. As a result, the WHO categorised them as global priority pathogens to guide research (Tacconelli et al., 2018).

In many SSA countries, including Mozambique, the burden and impact of AMR infections, particularly those associated with ESKAPE-E remain to be elucidated. To understand the epidemiological trends of bacterial AMR pattern in Mozambique, the Centro de Investigação em Saúde de Manhiça (CISM) has been conducting a 24h paediatric morbidity surveillance, including systematic blood samples collection among patients admitted at the Manhiça District Hospital-MDH (southern Mozambique) and Quelimane Central Hospital-QCH (central Mozambique, since 2023). Recent data of this surveillance ranked MDR *E. coli, S. aureus, Kp* among the leading pathogens in bacteraemia, associated with high

case fatality rates (e.g. 63% for *Kp*) (Sigaúque *et al*. 2009; Mandomando *et. al*, 2020; Garrine *et al.*, 2023). This was reinforced by data from the ongoing Child Health and Mortality Prevention Surveillance (CHAMPS) Network conducted by the CISM in the same settings, which identified *Kp* in the chain of deaths (21%, 497/2352) of deceased children under 5 years (Verani *et al*. 2024), along with *E. coli* and *S. aureus.*

Comparative analysis of *Kp* from paediatric patients and CHAMPS post-mortem revealed a predominance of MDR isolates harbouring ESBL genes (61% *vs.* 25%, *p*=0.001) encoded mainly by $bla_{\text{CTX-M-15}}$ (Massinga *et al.* 2021). Similarly, Garrine *et al.* (2023) reported the circulation of MDR *S. aureus* emergent clones (CC121 and CC152 by MLST) associated with mortality. Hence, to comprehensively grasp the landscape of AMR in Mozambique, it is imperative to investigate the interplay between bacterial strains and their genomic environment, particularly the AMR genes acquisition. Therefore, we intended to conduct genomic surveillance of our >25 years ESKAPE-E isolates, capitalizing the recently installed Illumina MiSeq platform at CISM. With this, we will create local capacity on genomic surveillance and new insights in the epidemiology of these global priority pathogens. Additionally, our data will inform prevention and control strategies, and potential vaccine development. In the future, these capacities will be used in the surveillance of other pathogens with epidemic potential underexplored in our country (e.g. *Salmonella* Typhi, *Vibrio cholerae*).

2.3. Narrative

Please describe the specific objectives, the approach, and the expected outputs and outcomes of the project (500 words max).

For this project we have defined the following specific objectives:

1. To improve the understanding of the burden and impact of AMR in the dynamics of its emergence and morbi-mortality associated through insights from genomic data in a low resource setting with limited therapeutic options.
2. To investigate the genetic diversity, resistance patterns and virulence profiles of *Kp* and other ESKAPE-E associated with AMR in Mozambique using whole genome analysis.
3. To strengthen local human resources capacities by conducting genomic surveillance using state-of-the-art technologies (Next Generation Sequencing and Bioinformatics) and to promote leadership and collaborations to respond to local surveillance, research and programmatic needs.

CISM recently acquired and installed an Illumina MiSeq sequencing platform to initiate genomic surveillance of malaria. However, this NGS platform has not been fully utilized for research and surveillance in clinical microbiology due to the absence of trained personnel and specific resources required to establish it as a standard method. Therefore, to achieve the objectives indicated above, we will leverage the collaboration we have with the International Centre for Reference and Research on *Escherichia, Shigell*a and *Klebsiella* in the Statens Serum Institut, Denmark, which is a reference in the sequencing of *Enterobacteriaceae* and enteroaggregative *Escherichia coli* (EAEC) in particular. This collaboration will provide technical support to CISM's team by assessing the needs at the local laboratory to help establish an implementation plan to proceed with the sequencing of bacterial pathogens locally. We plan to initiate sequencing and training using *K. pneumoniae* strains, followed by other ESKAPE-E pathogens. These strains have been collected and stored for over 25 years through our IBD surveillance in the Manhiça district. These activities will produce two main outputs: (1) the detailed characterization (genetic diversity, virulence and AMR pattern) at the genomic level of pathogens causing IBD over the past two decades, which have the potential to inform health policy, such as the design and development of interventions (change in the empiric therapy, vaccines, new drugs, etc.); (2) establish the capacity to conduct genomic surveillance, which is invaluable to ensure readiness to respond to any potential health emergency. Additional outputs within this project are to train postgraduate students, who will get mentorship and use the data generated to complete their

academic degrees. They will be involved in the data analysis and publication, allowing the project to increase the critical mass of Mozambican researchers.

This project falls within priority #22 of the WHO's Global Research Agenda for Antimicrobial Resistance in Human Health, under the "Cross-cutting priorities: Antimicrobial resistance epidemiology, burden and drivers" (WHO 2023) which specifies the investigation of the prevalence, incidence, mortality, morbidity and socioeconomic impact of community acquired infections and healthcare-associated infections by resistant WHO bacterial priority pathogens, across socioeconomic settings, especially in low- and middle- income countries, such as Mozambique.

3. Resource requirements

Please indicate estimates for the proposed duration and budget request. A revised duration and a detailed budget will be requested if the proposal is shortlisted for a full application.

Proposed duration (in months)	
Budget request (in USD)	☐ 0 ☐ 50,000-149,999 ☒ 150,000-250,000
Previous sources of funding for this project (please include amount in USD and funders name)	No previous sources of funding for this project
Current sources of funding for this project (please include amount in USD and funders name)	No other sources of funding are currently available for this project
Anticipated sources of funding for this project (please include amount in USD and funders name)	No other sources of funding have been confirmed for this project, even though we plan to request for funding from other sources

4. Eligibility

Does the applicant organization(s) agree to open access regarding project results that enables the unrestricted access and reuse of all peer-reviewed published research? If no, please explain.	☒ Yes ☐ No
Does the proposal include research involving human participants or individual data?	☒ Yes ☐ No
If the proposal involves individual data, is the applicant organization(s) equipped to collect, anonymize, disseminate, and protect individual data ethically? *Any research involving human participants and/or individual data will be subject to additional ethical review.*	☒ Yes ☐ No ☐ Not applicable
Applicant organization(s) agrees to undergo due diligence in line with the UN Foundation and WHO regulations and guidelines and submit additional disclosures upon request.	☒ Yes ☐ No
Are any of the applicant organization(s) in this proposal also applying for other grants (individually or in consortium) in this funding round?	☐ Yes: Please provide additional context (max 100 words) _____ ☒ No