



Newton Agham Fund

PROONENTS:

Celia Carlos^{1,2}, David Aanensen²

TEAM MEMBERS:

Sonia Sia¹, Charmian Hufano^{1,2}, Marietta Lagrada¹, Agnethali Olorosa¹, Polle Krystle Macaranas¹, June Gayeta¹, Melissa Ana Masim¹, Marilyn Limas¹, Manuel Jamoralin¹, Holly Grace Espiritu¹, Jaywarddeen Abad, Janziel Fiel Palarca¹, Jeremiah Chilam¹, Alfred Villamin¹, June Janice Borlasa¹, Mariane Magbanua¹, Karis Boehmel¹, Lara Fides Hernandez¹, Silvia Argimon², Victoria Cohen², Benjamin Jeffrey², Khalil Abudahab², John Stelling⁴, Matthew Holden⁵

^aCONTACT DETAILS

Research Institute for Tropical Medicine
Alabang, Muntinlupa, Philippines
+63 8807 2628 | ccarlosph@gmail.com

AFFILIATIONS

¹Research Institute for Tropical Medicine, Alabang, Muntinlupa, Philippines

²Centre for Genomic Pathogen Surveillance, Wellcome Trust Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom

³St. Luke's Medical Center – Bonifacio Global City, Taguig, Philippines

⁴Brigham and Women's Hospital, Boston, MA, USA

⁵University of St Andrews School of Medicine, St Andrews, Scotland, UK

See and Sequence: Genomic surveillance of antimicrobial resistant and high-risk pathogenic clones

ABSTRACT

National networks of laboratory-based surveillance of antimicrobial resistance monitor resistance trends and disseminate these data to AMR stakeholders. Whole-genome sequencing (WGS) can support surveillance by pinpointing resistance mechanisms and uncovering transmission patterns. However, genomic surveillance is rare in low- and middle income countries. Here, we implement WGS within the established Antimicrobial Resistance Surveillance Program of the Philippines via a binational collaboration. In parallel, we characterize bacterial populations of key bug-drug combinations via a retrospective sequencing survey. By linking the resistance phenotypes to genomic data, we reveal the interplay of genetic lineages (strains), AMR mechanisms, and AMR vehicles underlying the expansion of specific resistance phenotypes that coincide with the growing carbapenem resistance rates observed since 2010. Our results enhance our understanding of the drivers of carbapenem resistance in the Philippines, while also serving as the genetic background to contextualize ongoing local prospective surveillance.

Abstract from: Argimon, S., Masim, M. A., Gayeta, J. M., Lagrada, M. L., Macaranas, P. K., Cohen, V., ... & Carlos, C. C. (2020). Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines. *Nature*

OBJECTIVE

Existing surveillance networks to detect increasing antimicrobial resistance need new tools to enable a more detailed understanding of where and when AMR is arising across a region. We aimed to establish local capacity and expertise for whole genome sequencing for pathogen surveillance in the Philippines, which would allow increased resolution when identifying and characterizing high-risk bacterial lineages important in public health. This would also enable targeted monitoring of high-risk clones to prevent, control and limit their spread.

METHODOLOGY

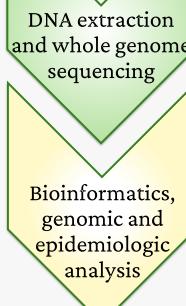
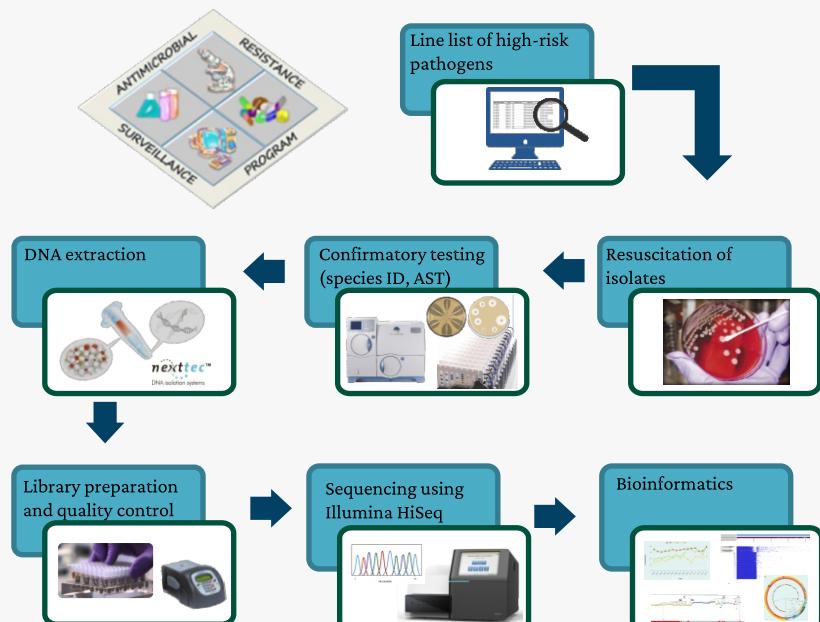


- Biosurveillance
- Bioinformatics (assembly, annotation, variant calling, and phylogenetic analysis)
- Bacterial DNA extraction, DNA library preparation, and genome sequencing
- Short reports write shop

Figure 1. Trainings of ARSRL staff in laboratory and bioinformatic procedures for capacity-building. (a) Planning workshop led by Dr David Aanensen to discuss methods in obtaining baseline data for local prospective sequencing. (b) Workshop on bioinformatics and genome analysis at PGC. (c) Training on bioinformatics, phylogenetics, molecular epidemiology, and sequence data handling at the Centre for Genomic Pathogen Surveillance (CGPS). (d) Hands-on training on bacterial DNA extraction and genome sequencing at the Philippine Genome Center. (e) DNA extraction and sequencing library training at CGPS, (f) supervised by Dr Michael Quail of Sanger Institute.



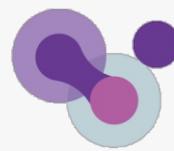
LABORATORY AND DATA ANALYSIS WORKFLOW



- Retrospective selection of 2013-2014 isolates
 - S. aureus*, *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *N. gonorrhoeae*, *Salmonella Typhi*, Non-Typhoidal *Salmonella*
- Data integration into WHONET

- Extracted DNA were sent to Wellcome Trust Sanger Institute for sequencing using Illumina HiSeq platform

- Genomic characterizations
- Identification of mechanisms of resistance
- Phylogenetic analysis
- Retrospective detection of outbreaks



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RESULTS

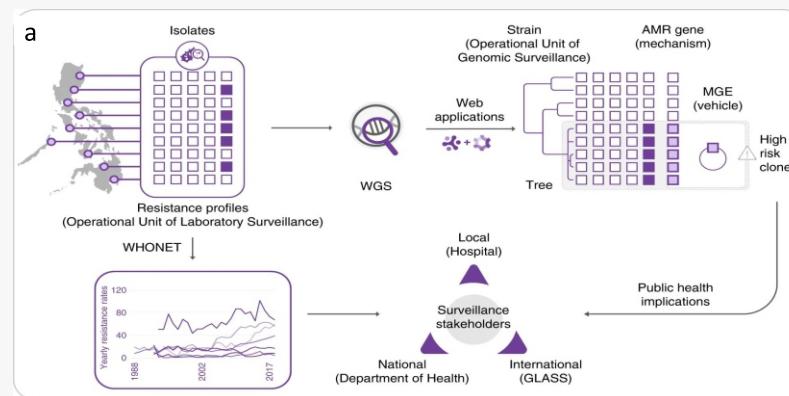


Figure 2. Integrating whole genome sequencing (WGS) in the national antimicrobial resistance surveillance. (a) Isolates collected by sentinel sites are tested for antimicrobial susceptibility. The data are stored as resistance profiles in WHONET and summaries of resistance trends are shared yearly with surveillance stakeholders. WGS of bacterial isolates and interpretation with web applications like Microreact and Pathogenwatch provide information on genetic relatedness (strains), known AMR determinants (mechanisms), and the mobile genetic elements (MGE, vehicles) for their dissemination, thus allowing us to detect high-risk clones.

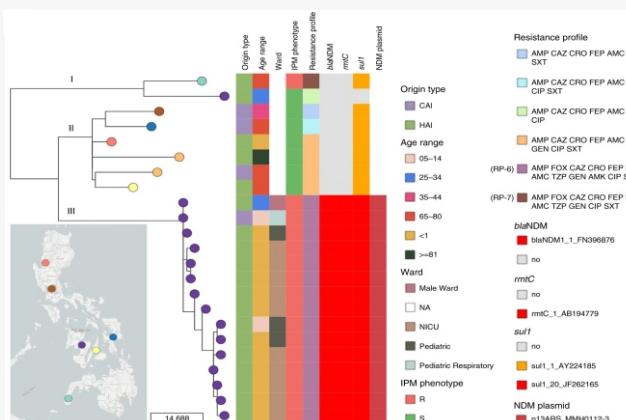


Figure 3. A plasmid-driven outbreak of *K. pneumoniae* ST340 was detected using WGS analysis. Maximum likelihood tree of 24 retrospective ST340 genomes inferred from 196 SNP positions identified by mapping the genomes to reference CAV1217 (CP018676.1), and masking regions corresponding to mobile genetic elements and recombination. In the linked epidemiological and genotypic data, origin type is defined as either community-acquired infection (CAI) or hospital-acquired infection (HAI). NA ward information not available. The imipenem (IPM) phenotype was either resistant (R) or susceptible (S). The scale bar shows the number of SNPs per variable site.

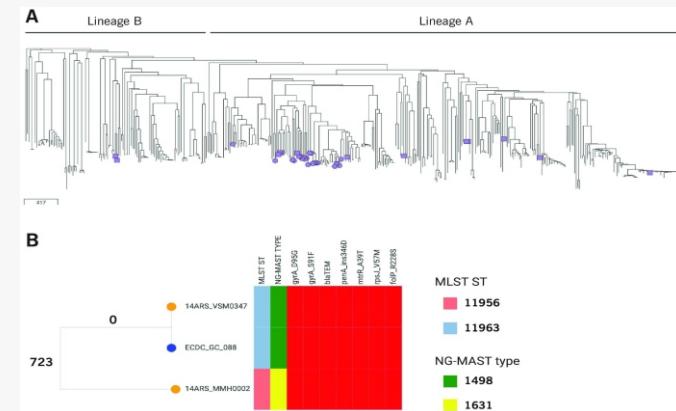


Figure 4A. Phylogenetic tree of 416 Philippine *N. gonorrhoeae* (circles) against global collections (squares) from Pathogenwatch shows their multidrug-resistant lineage. The tree is inferred from 22,558 variant sites in 1,542 core genes. The scale bar represents the number of SNPs. **B. Subtree of closely related Philippine (orange nodes) and Norway (blue) isolates.** The tree branches are annotated with the number of pairwise SNP differences between isolates. The metadata blocks indicate the ST, NG-MAST type and the presence (red blocks) of seven AMR determinants.

PUBLICATIONS

1. Argimón, S., Masim, M. A., Gayeta, J. M., Lagrada, M. L., Macaranas, P. K., Cohen, V., ... & Carlos, C. C. (2020). Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines. *Nature communications*, 11(1), 1-15.
 2. Masim, M. L., Argimón, S., Espiritu, H. O., Magbanua, M. A., Lagrada, M. L., Olorosa, A. M., ... & Carlos, C. C. (2021). Genomic surveillance of methicillin-resistant *Staphylococcus aureus* in the Philippines, 2013–2014. *Western Pacific surveillance and response journal: WPSAR*, 12(1), 6.
 3. Jamoralin Jr, M. C., Argimón, S., Lagrada, M. L., Villamin, A. S., Masim, M. L., Gayeta, J. M., ... & Carlos, C. C. (2021). Genomic surveillance of *Neisseria gonorrhoeae* in the Philippines, 2013–2014. *Western Pacific Surveillance and Response Journal: WPSAR*, 12(1), 17.
 4. Chilam, J., Argimón, S., Limas, M. T., Masim, M. L., Gayeta, J. M., Lagrada, M. L., ... & Carlos, C. C. (2020). Genomic Surveillance of *Pseudomonas aeruginosa* in the Philippines from 2013–2014. *bioRxiv*.
 5. Chilam, J., Argimon, S., Limas, M. T., Masim, M. L., Gayeta, J. M., Lagrada, M. L., ... & Carlos, C. C. (2021). Genomic Surveillance of *Acinetobacter baumannii* in the Philippines, 2013–2014. *bioRxiv*.
 6. Antimicrobial Resistance Surveillance Reference Laboratory. (2019). Concordance of Traditional Serotyping and Whole Genome Sequencing of Non-Typhoidal *Salmonella* Isolates in the Philippines, Years 2013–2014. In *55th Annual Meeting of the International Society for Microbiology*.



Figure 5. The ARSRL staff with some of the UK collaborators during a visit to the Philippines