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See and Sequence: Integrating Whole-Genome Sequencing Within the National Antimicrobial Resistance Surveillance Program in the Philippines

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Abstract

Background. Drug-resistant bacterial infections constitute a growing threat to public health globally. National networks of laboratory-based surveillance of antimicrobial resistance (AMR) monitor the emergence and spread of resistance and are central to the dissemination of these data to AMR stakeholders. Whole-genome sequencing (WGS) can support these efforts by pinpointing resistance mechanisms and uncovering transmission patterns. We implemented WGS within the established Antimicrobial Resistance Surveillance Program (ARSP) of the Philippines. We aimed to employ WGS to characterize bacterial populations and dissect resistance phenotypes of key bug-drug combinations, thus establishing a genetic background to contextualize local prospective surveillance.

Methods. We sequenced the genomes from eight bacterial pathogens collected between 2013 and 2014 by the ARSP, and conducted phylogenetic analyses, *in silico* genotyping, genomic predictions of AMR, and characterization of key plasmids carrying carbapenemase genes. Here, we focus on carbapenemase-producing organisms.

Findings. ARSP phenotypic data indicated increasing carbapenem resistance for *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli*, with marked expansion of specific resistance phenotypes. By linking resistance phenotypes to genomic data, we revealed the diversity of genetic lineages (strains), AMR mechanisms, and AMR vehicles underlying this expansion. We discovered a previously unidentified plasmid-driven hospital outbreak of carbapenem-resistant *K. pneumoniae*, uncovered the interplay of carbapenem resistance genes and plasmids in the geographic circulation of international epidemic *K. pneumoniae* ST147, and found that carbapenem-resistant *E. coli* ST410 were represented by diverse lineages of global circulation that both conserved international plasmids and acquired plasmids of local circulation.

Conclusions. WGS provided an enhanced understanding of the interplay between strains, genes and vehicles driving the dissemination of carbapenem resistance in the Philippines. We generated a blueprint for the integration of WGS and genomic epidemiology into an established national system of laboratory-based surveillance of AMR through international collaboration. Continued prospective sequencing, capacity building and collaboration will strengthen genomic surveillance of AMR in the Philippines and the translation of genomic data into public-health action.

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Introduction

Antimicrobial resistance (AMR) is an increasingly serious threat to global public health and economy that requires concerted action across countries, government sectors and non-government organizations (1). Without AMR containment, an adverse impact on public health costs, global gross domestic product (GDP), livestock production and international trade is expected by 2050, and the sustainable development goals for 2030 are less likely to be attained (2). The Global Action Plan developed by the World Health Organization (WHO) to tackle AMR highlights the need to strengthen our understanding of how resistance develops and spreads, and the underlying resistance mechanisms (3). One of the pillars of this objective is the national surveillance system for antimicrobial resistance.

An advanced example of a national surveillance system is the Antimicrobial Resistance Surveillance Program (ARSP), the national laboratory-based surveillance of the Philippine Department of Health (DOH). The ARSP began in 1988 with ten tertiary hospitals (sentinel sites) and the Antimicrobial Resistance Surveillance Reference Laboratory (ARSRL) in Metro Manila and has since expanded to 25 sentinel sites and two gonorrhoea surveillance sites in all 17 regions of the country (Supplementary Figure S1). Surveillance encompasses common pathogens of public health importance in the Philippines, where infectious diseases represented nine out of the ten leading causes of morbidity (4), and four out of the ten leading causes of infant mortality (5) in 2013-2014. Results of culture-based identification and antimicrobial susceptibility are stored centrally at the ARSRL within the clinical microbiology management software WHONET (Figure 1A, (6)). A report summarizing the resistance trends is published annually and disseminated to local, national and international surveillance stakeholders (7). For policy change, surveillance data generated by ARSP is used by the DOH to develop clinical practice guidelines and determine the panel of antibiotics to be included in the national formulary. Furthermore, ARSP has been contributing data to international AMR surveillance programs since the 1980s, including the Global Antimicrobial Surveillance System (GLASS (8)) since 2015. Importantly, the ARSP data has informed the Philippine National Action Plan to combat AMR, one of the key requirements for alignment with the WHO global action plan.

The Philippines has seen a steady increase in resistance rates for several key pathogen-antibiotic combinations (Figure 1B), including carbapenem-resistant organisms in the last 10 years. The genetic mechanisms underlying carbapenem resistance, one of the biggest therapeutic challenges in the treatment of antimicrobial resistant infections, include increased upregulation of efflux pumps, decreased uptake by altered expression/loss of porin function, and acquisition of hydrolytic enzymes –carbapenemases (9). Whole-genome sequencing (WGS) of bacterial pathogens can identify distinct clonal lineages (strains) on phylogenetic trees, known AMR mechanisms (genes or mutations) and the vehicles for the acquired AMR genes (mobile genetic elements, MGEs) which, in turn, enables enhanced detection and characterization of high-risk clones (Figure 1A). WGS has improved outbreak investigations and epidemiological surveillance (10, 11), and enhanced our knowledge of the spread of antimicrobial-resistant strains and their resistance mechanisms (12, 13). Elucidation of resistance mechanisms and their context can be critical for effective infection control. For example, upregulation of efflux pumps and loss of porin function by mutation are vertically transmitted, while acquired carbapenemases carried in transmissible plasmids or integrative conjugative elements have the potential for horizontal dissemination between strains and species, thus necessitating enhanced infection control measures (14). Moreover, diverse

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carbapenemase classes, variants, flanking MGEs, and associated plasmid incompatibility groups have been described, requiring multiple biochemical and molecular tests for identification. For example, the class B New Delhi metallo-beta-lactamase (NDM) is found worldwide, represented by over ten different variants that are usually upstream of intact or truncated IS*Aba125* elements, within plasmid backbones of different incompatibility groups, across multiple lineages of *E. coli* –as well as of other carbapenem-resistant organisms (15, 16). WGS can determine the interplay between these different components, i.e. the gene, the vehicle, and the strain, thus maximizing the epidemiological benefit derived from its cost.

Implementation of WGS within existing national surveillance systems in LMICs has the potential to enhance local prevention and control of resistant infections in a sustainable and equitable manner. Integration of WGS into routine surveillance of AMR can be facilitated by international collaboration focused on transfer of expertise and ownership. Our collaboration (See and Sequence, Figure 1A) aimed to implement WGS within the ARSP via a multi-faceted approach that included a large initial retrospective sequencing survey, technology transfer, utilization of user-friendly web applications, and capacity building in laboratory and bioinformatic procedures for local prospective sequencing. We linked what has traditionally been used as the operational unit of laboratory surveillance, the resistance profile, with the operational unit of genomic epidemiology now provided by WGS, genetic relatedness. Here we provide exemplars that highlight how the granular view of strain-gene-vehicle in carbapenem-resistant populations at the local, national and global operational scales can be leveraged for surveillance of AMR and public health.

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Methods

ARSP data and bacterial isolates included in this study

Phenotypic data collected since 1988 from antimicrobial susceptibility tests (Supplementary Methods, Appendix) was summarized with WHONET to compute yearly resistance rates for key pathogen-antibiotic combinations. Only the first bacterial isolate per patient per species per year was included in the calculations.

Resistance profiles derived from the antibiograms were summarized per organism with WHONET, and their relative abundance visualized with Tableau Desktop. Data collected between 2009 and 2017 were included in the analysis as the number of sentinel sites collecting data remained relatively stable during this period (22-24 sites). Isolates with missing data for any antibiotic on the panels listed on Table 1 were excluded.

Isolates of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and carbapenemase-producing or ESBL-suspected *Escherichia coli* and *Klebsiella pneumoniae* collected in 2013 and 2014 confirmed by the ARSRL were selected for sequencing (Supplementary Methods, Appendix). Linked epidemiological data included location and date of specimen collection, type of specimen, type of patient (in or outpatient), and sex and age of the patient. We utilized a proxy definition for “infection origin” whereby patient first isolates collected in the community or on either of the first two days of hospitalization were categorized as community infection isolates, while isolates collected on hospital day three or later were categorized as hospital-acquired infection isolates (8).

Whole Genome Sequencing Methods and Analyses

DNA was extracted from a single colony of each isolate of *A. baumanni*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* with the QIAamp 96 DNA QIAcube HT kit and a QIAcube HT (Qiagen; Hilden, Germany). DNA extracts were multiplexed and sequenced on the Illumina HiSeq platform (Illumina, CA, USA) with 100-bp paired-end reads. Select isolates were also sequenced with PacBio RS II, Sequel (Pacific Biosciences, CA, USA) or Oxford Nanopore Gridion (Oxford Nanopore, Oxford, UK) platforms, and their plasmids were characterized (Supplementary Methods, Appendix). Raw sequence data were deposited in in the European Nucleotide Archive (ENA) under the study accession no. PRJEB17615. Individual accession numbers for raw sequence data and samples are available from the linked Microreact projects.

We detected the presence of known AMR determinants and plasmid replicons in the whole genomes and inferred phylogenetic trees from single-nucleotide polymorphisms (SNPs) identified in reference-based genome alignments. We also derived *in silico* the multi-locus sequence typing (MLST) sequence types (STs) (Supplementary Methods, appendix). Where appropriate, the Philippine genomes were contextualized with global genomes available at the ENA. Phylogenetic trees, genomic predictions of AMR, and genotypic results were visualised together with the ARSP epidemiological data using Microreact (17). The combined distributions of resistance profiles, STs, and resistance genes were visualized with Tableau Desktop.

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Results

The Philippine Antimicrobial Resistance Surveillance Program (ARSP) collects bacterial isolates and stores the associated clinical and epidemiological data in the WHONET software. Antibiograms are stored as resistance profiles, which represent the diversity of AMR phenotypes in the country. In parallel to local WGS capacity building, we conducted a large retrospective sequencing survey of eight bacterial pathogens to provide genomic context for local prospective surveillance. Here, we focus on the carbapenem-resistant organisms, which have been classified as of critical priority for the development of new antibiotics by the World Health Organization (WHO).

Operational unit of laboratory surveillance - Routine laboratory surveillance highlights the increasing burden of specific resistance phenotypes

Carbapenem-resistant *P. aeruginosa, A. baumannii, K. penumoniae* and *E. coli* were all first isolated by ARSP between 1992 and 1994. Since the year 2000 resistance rates to imipenem and meropenem have remained below 30% for all organisms except for *A. baumannii*, which has seen a steady rise in resistance rates between 2009 and 2017, reaching 56% (Figure 2A). This coincides with the expansion of two resistance profiles (RP), first a striking expansion of true-XDR and possible-PDR RP-1, and later of true-XDR RP-2 (Figure 2B). Yearly resistance rates for *P. aeruginosa* have oscillated between 10-25% since the year 2000, and have doubled between 2010 and 2013 (Figure 2A). Two resistance profiles have seen the largest combined expansion between 2011 and 2013, possible-PDR RP-3, and true-XDR RP-4 (Figure 2B). The yearly resistance rates to imipenem for Enterobacteriaceae were approximately 3% in 2011 but have since steadily increased to 4·8% for *E. coli* and 10·9% for *K. pneumoniae*, respectively (Figure 2A). This coincides with the expansion of three possible-XDR resistance profiles, RP-5, RP-6, and RP-7 (Figure 2B). The observed expansion of specific resistance profiles could be driven by the rapid dissemination of a discrete genetic lineage (strain) carrying AMR determinants or, alternatively, of transmissible MGEs, which shuttle resistance determinants across different genetic lineages.

Operational unit of genomic surveillance - WGS reveals the genetic diversity and AMR mechanisms underpinning carbapenem resistance phenotypes

The sequences of 805 genomes linked to WHONET data were obtained from isolates belonging to the four bacterial pathogens in the WHO critical list (Table 1), which were collected in 2013 and 2014 by between seven to eighteen ARSP sites. The isolates sequenced were biased towards the key resistances listed in Table 1, with the exception of *P. aeruginosa*, for which susceptible isolates were also included.

The distribution of the carbapenem resistance profiles highlighted in Figure 2 was not concordant with the major clades observed on the phylogenetic trees (Figure 3). Instead, the same resistance profile was usually observed in multiple genetic lineages across the tree. Yet, the distribution of the sequence types (STs) inferred from the genome sequences was largely concordant with the major clades observed on the trees (Figure 3), confirming that they represent well-defined genetic lineages. This largely extends to all carbapenem resistance profiles in our dataset, as the number of ST assignments increases with the number of isolates representing a resistance profile, both at the national and local levels (Supplementary Figure S2). Thus, the genomic data suggests that the expansion of specific carbapenem resistance profiles is driven by the dissemination of

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resistance determinants *en-bloc* via MGEs, as previously observed for acquired carbapenemases (16).

Carbapenemases are a diverse collection of hydrolytic enzymes (16), and we identified representatives of class A, B and C carbapenemases in the Philippines (Supplementary Figure S3). Class B NDM-1 and Verona integron-borne metallo-beta-lactamase VIM-2 were the most prevalent and geographically disseminated in the Enterobacteriaceae and *P. aeruginosa,* respectively, while the same was true for class D OXA-23 in *A. baumannii*. Importantly, different carbapenemase genes/variants (or combinations thereof) were found underlying the same resistance profiles in all four organisms (Figure 3).

Operational scale of surveillance – Local: WGS reveals a plasmid driven hospital outbreak of carbapenem-resistant K. pneumoniae

Fifty-seven percent (N=33) of the *K. pneumoniae* genomes with the possible-XDR resistance phenotype “AMP FOX CAZ CRO FEP IPM AMC TZP GEN AMK CIP SXT” (RP-6) were isolated from a single hospital (MMH) but belonged to 12 different STs, with almost half of them (N=15) placed within ST340 (Supplementary Figure S4A). Phylogenetic analysis of these 15 genomes in the wider context of ST340 indicated three main lineages, with the 15 possible-XDR isolates from MMH forming a tight cluster (clade III, Figure 4A). The shorter branch lengths in the imipenem-resistant clade III (6.5±3.7 pairwise SNP differences) compared to the imipenem-susceptible clade II (26.8 ±3.7 pairwise SNP differences, Mann-Whitney U test z-score 6.245, p-value < 0.00001, Supplementary Figure S4B), and the isolation dates spanning twelve months, suggest a rapid expansion of this clone, which coincides with the acquisition of the *bla*$_{\text{NDM-1}}$ gene (Figure 4A).

Epidemiological data showed that the samples were mostly hospital-acquired (N=14), and from neonates (N=12) (Figure 4A). These observations triggered a retrospective epidemiological investigation that revealed that 10 of the isolates originated from patients of the neonatal intensive care unit (NICU), all of which had umbilical catheters and were on mechanical ventilators. The remaining cases were either from paediatric wards (N=4) or the male ward (N=1), suggesting wider dissemination of this high-risk clone within the hospital environment.

The genetic diversity underlying the 33 possible-XDR isolates from MMH (12 STs) prompted us to investigate the hypothesis of dissemination of carbapenem resistance in the hospital by a plasmid carrying *bla*$_{\text{NDM-1}}$. We identified a 101,540 bp IncFII plasmid with *bla*$_{\text{NDM-1}}$, *rmtC*, and *sul1* (p13ARS_MMH0112-3, Table 2 and Supplementary Results and Figure S5) in a representative isolate from ST340 clade III. In addition, we found the entire p13ARS_MMH0112-3 plasmid sequence (i.e. ≥95% of the length) represented in 27 genomes from 9 different sequence types, including 20 samples from 6 different sequence types isolated from patients under 1 year old (Figure 4B).

Altogether, our findings suggest that the burden of carbapenem-resistant *K. pneumoniae* infections in hospital MMH was largely linked to plasmid p13ARS_MMH0112-3 with *bla*$_{\text{NDM-1}}$, circulating within diverse genetic lineages, and leading to outbreaks in high-risk patient populations. Hospital authorities were informed of these findings, and measures for infection control were implemented, including designating a separate multi-drug resistance organism (MDRO) room for cohorting, active surveillance upon identification of any new carbapenem-resistant *K. pneumoniae* from the NICU, and referral of any new carbapenem-resistant *K. pneumoniae* from the NICU to ARSRL for sequencing.

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Operational scale of surveillance - National: WGS reveals the interplay of carbapenem resistance genes and plasmids in the regional circulation of a successful K. pneumoniae *lineage*

The possible-XDR resistance phenotype “AMP FOX CAZ CRO FEP IPM AMC TZP CIP SXT” (RP-5) was represented by 76 *K. pneumoniae* isolates from 14 different STs. Seventy-one percent (N=54) belonged to ST147, an international epidemic clone that was found in 11 sentinel sites in all 3 island groups in the Philippines. The phylogeny of the ST147 genomes showed that carbapenem non-susceptible isolates (78·8%, N=63) were found in clades arising from 3 out of 4 deep branches of the tree (Figure 5A), which represent separate groups of the global population (Supplementary Figure S6A). Carbapenem resistance coincided with the presence of *bla*$_{\text{NDM-1}}$ in clade IV genomes, while clade III-B shows geographically distinct clusters with either *bla*$_{\text{NDM-1}}$ or *bla*$_{\text{NDM-7}}$ (Figure 5A and Supplementary Results and Figure S7).

Plasmid sequences obtained from isolates representing different carbapenem-resistant clusters in clades III and IV showed that the NDM genes were carried within different variants of the insertion sequence IS*Aba*125 on different plasmid backbones, two of which showed high sequence similarity to international plasmids (Table 3 and Supplementary Figures S5B, S6B and S6C). The distribution of plasmids harbouring *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{NDM-7}}$ matched the strong phylogeographic signal in the terminal branches of the tree (Figure 5A). Within clade III-B, a cluster of genomes from sentinel sites MMH and STU were characterized by plasmid p14ARS_MMH0055-5 carrying *bla*$_{\text{NDM-7}}$, while another cluster of genomes from VSM and NMC were distinguished by plasmid p13ARS_VSM0593-1 with *bla*$_{\text{NDM-1}}$. Plasmids p13ARS_GMH0099 and p14ARS_VSM0843-1, both carrying *bla*$_{\text{NDM-1}}$ were found in different subclades of clade IV, one of local circulation in hospital VSM, and another one showing regional expansion across 5 sentinel sites (Figure 5A). Taken together, the phylogeographic signal (strains) in combination with the distribution of *bla*$_{\text{NDM}}$ variants (mechanism) and plasmids (vehicle), revealed local and regional patterns of dissemination of carbapenem non-susceptible ST147 within the Philippines, which could not be identified based solely on the resistance profiles or MLST information. Furthermore, comparison with global ST147 genomes indicated that clade IV may be endemic to the Philippines (Supplementary Results and Figure S6A).

Operational scale of surveillance - International: First report of a high-risk clone of E. coli *ST410 carrying bla*$_{\text{NDM-1}}$ *and bla*$_{\text{OXA-181}}$

Recent reports of *E. coli* ST410 international high-risk clones carrying class D and class B carbapenemases (18, 19) are particularly alarming in light of their wide geographic distribution and the broad variety of niches this clone can occupy (20, 21). ST410 was the second most prevalent ST in the retrospective collection of *E. coli* (13·2%, N=24), encompassing a large proportion of imipenem-resitant isolates (N=15), and the three expanding possible-XDR resistance profiles RP-5,6,7 (Supplementary Figure S8). The phylogenetic tree of 24 ST410 genomes showed that isolates with the possible-XDR profiles clustered within one clade (Figure 6A), which can be further delineated by the distribution of carbapenemase genes and variants into three different clones carrying *bla*$_{\text{NDM-4}}$ (N=1), *bla*$_{\text{NDM-1}}$ (N=8), or both *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$ (N=5). The phylogenetic analysis with global *E. coli* ST410 genomes confirmed that these are independent clones, as they were found interspersed with international genomes within a major high-risk lineage (Figure 6B and Supplementary Results).

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K. pneumoniae carrying *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$ have been previously reported (22, 23), as well as *E. coli* ST410 harbouring *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$ from Egypt (24), Denmark, and the UK (19), but, to our knowledge, this is the first report of *E. coli* carrying both *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$, which is liekely to have disseminated between two sentinel sites (NMC and VSM). We identified five plasmids in the representative strain 14ARS_NMC0074 (Table 3, and Supplemental Figure S9). The IncX3 plasmid carrying *bla*$_{\text{OXA-181}}$ (p14ARS_NMC0074-5) was identical to plasmid pAMA1167-OXA-181 isolated previously from an *E. coli* ST410 strain (25) (Supplemental Figure S9). Mapping short reads to p14ARS_NMC0074-5 showed that this plasmid is the main vehicle of *bla*$_{\text{OXA-181}}$ in the Philippines, as in the international genomes (Figure 6B, (19)). However, we did not identify any plasmids similar to the IncA/C2 plasmid with *bla*$_{\text{NDM-1}}$ (p14ARS_NMC0074-2), though it shared approximately 90% of its backbone with the IncA/C2 plasmid described above from *K. pneumoniae* ST147 strain 13ARS_VSM0593 (Supplemental Figure S9). This plasmid backbone was found in *E. coli* ST410 genomes from the Philippines, but not in international genomes (Figure 6B).

Altogether, our results show that that the Philippine *E. coli* ST410 genomes represent diverse lineages of the global circulating population, with evidence of frequent international transmission. These lineages are characterized by a diverse repertoire of carbapenemase genes and variants, amassed via a combination of conserved international plasmids and locally-circulating plasmids.

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Discussion

National networks of laboratory-based surveillance are a key pillar within the Global Action Plan to combat AMR, with the reference laboratory playing a central role in the dissemination of surveillance data to local, national and international stakeholders. The Phillipines ARSP surveillance data has clearly shown increasing resistance trends for key bug-drug combinations (Figures 1B and 2A), and the expansion of specific resistance phenotypes (Figure 2B). By complementing laboratory data with WGS and linking the operational units of laboratory and genomic surveillance, we have revealed a diversity of genetic lineages, AMR mechanisms, and vehicles underlying the expansion of carbapenem resistance phenotypes (Figures 1A and 3).

A crucial aspect of AMR surveillance is the detection of the emergence and spread of high-risk clones. In traditional laboratory surveillance, resistance profiles can be analysed with spatiotemporal algorithms to detect hospital outbreaks (26) or dissemination of phenotypic subpopulations between hospitals (27). Cluster detection based on resistance profiles depends on consistent and complete antibiograms within and across sentinel sites. Indeed, our project highlighted the need to reinforce the standardization of antibiotic testing across the ARSP sentinel sites, which was then coordinated by the ARSRL.

Even with complete and comprehensive susceptibility testing, resistance profiles can only provide a coarse view of the spread of AMR, and sometimes lead to clusters of disparate genetic relatedness (Figure 3 and Supplementary Figure 2). Integration of WGS into laboratory-based AMR surveillance can substantially improve the detection of high-risk clones by providing a high-resolution picture of genetic lineages (strains), AMR mechanisms (genes/mutations) and vehicles (MGEs, Figure 1A). We identified high-risk clones within known international epidemic clones at the local, national, and international scales by linking clonal relatedness, geographic clustering, epidemiological data, and gene and plasmid content with interactive web tools (17).

At the local scale, we identified a plasmid-driven NICU outbreak of carbapenem-resistant *K. pneumoniae*. The epidemiological data captured in WHONET was key to support the interpretation of the genomic findings, triggering a retrospective investigation. This previously undetected outbreak was traced to an IncFII plasmid carrying $bla_{\text{NDM-1}}$ in the genetic context of ST340 (Figure 4A). Yet, the endemic IncFII plasmid was found across multiple wards, and genetic backgrounds (STs, Figure 4B), indicating a major role in the burden of carbapenem resistance *K. pneumoniae* in this hospital. A second IncFIB-IncFII plasmid found only within the ST340 lineage might have contributed to the persistence and transmission of this clone, in particular within the NICU, by carrying several genomic islands with a role in survival in the host and in the environment (Supplementary Figure S5). ST340 is a member of the drug-resistant clonal complex 258 and has been reported to cause outbreaks worldwide (28). Our findings were disseminated to the local stakeholders (i.e., hospital) via a forum with NICU staff, paediatricians and ARSRL representatives. Infection control strategies were implemented, which ultimately bolstered the infection control team of this hospital. Control of healthcare-associated infections is crucial to containing the spread of antimicrobial resistance (29). The roadmap from sequence data to actionable data for infection control and prevention within a hospital has been mapped by multiple use cases (30, 31) and supported by recent studies on implementation and cost-effectiveness in high-resource settings (32, 33). This study makes a case for extending this roadmap to LMICs that have infection control capacity in place.

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At the national scale, the combined information on strain-gene/variant-vehicle shows a granular picture of carbapenem-resistant *K. pneumoniae* ST147 that uncovers the geographic distribution of high-risk clones. ST147 is an international epidemic clone that causes carbapenem-resistant infections mediated by NDM, OXA-48-like and VIM carbapenemases (28, 34). However, ST147 was not reported in a recent study of *K. pneumoniae* from seven healthcare facilities across South and Southeast Asia (35), which did not include the Philippines. Within the seemingly endemic genetic background of lineage IV (Figure 5 and Supplementary Figure 7), a high-risk clone characterized by the presence of *bla*$_{\text{NDM-1}}$ within plasmid p14ARS_VSM0843-1 displayed local clonal expansion in one sentinel site, while another high-risk clone characterized by the presence of *bla*$_{\text{NDM-1}}$ within plasmid p13ARS_GMH0099 had also disseminated geographically across five different sites (Figure 5). Clonal expansion followed by geographical dissemination is usually attributed to the acquisition of an AMR determinant (36). The extent of the geographical dissemination of the different high-risk clones could be attributed to the different plasmid backbones (vehicle), but differences in the genetic background (strain) could also be at play. The presence of a robust AMR surveillance network such as the ARSP is key for the detection of geographical dissemination of high-risk clones at a regional/national level, and for the dissemination of the information to national stakeholders. The reference laboratory directors can alert hospitals across the country to establish concerted infection control measures, as well as relay this information to the national Department of Health for the formulation of evidence-based guidelines.

At the global scale, we identified several high-risk clones of *E. coli* ST410, with evidence of frequent international transmission, in agreement with a previous report (19). Previous reports of carbapenem-resistant *E. coli* ST410 from the Western Pacific and South East Asia regions described the presence of *bla*$_{\text{OXA-181}}$ in China (18, 37), *bla*$_{\text{NDM-1}}$ in Singapore (38), *bla*$_{\text{NDM-5}}$ in South Korea (39), or the combination of *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$ in Myanmar (40) and South Korea (39). The repertoire of carbapenemases in the retrospective collection of ST410 from the Philippines seemed to be in tune with the genes/variants circulating locally, as most isolates carried the prevalent variant *bla*$_{\text{NDM-1}}$. Of note was the high-risk clone carrying both *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$, a combination hitherto unreported in *E. coli* ST410, and acquired through the combination of a plasmid of local circulation and a conserved international plasmid, respectively. This suggests that *E. coli* ST410, a successful pandemic lineage, can not only easily disseminate, but it can also adjust the complement of carbapenemases by acquiring endemic plasmids along the way. Global AMR surveillance networks, such as GLASS (8), are paramount to detect the emergence and monitor the spread of resistance at the international level and inform the implementation of targeted prevention and control programmes.

Collecting information on AMR that can be rapidly transformed into action requires harmonized standards, especially at the national and international levels (8). For laboratory data, WHONET serves this purpose in the ARSP and in over 2,000 clinical, public health, veterinary, and food laboratories in over 120 countries worldwide (27), while GLASS serves this purpose at the global level. Genomic data is amenable to standardization (29), and national public health agencies (41) and international surveillance networks (42) are adopting diverse schema to identify and name genetic lineages. However, a global standard system for defining a cluster has not been implemented beyond the level of discrimination of MLST. Likewise, different databases of AMR mechanisms (43-45) may differ in content and nomenclature. Thus, the standardization of genomic data, as well as the provision of platforms for the uptake of genomic information are crucial moving forward, and for ongoing AMR surveillance.

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Containment of AMR at a global level requires an international concerted effort. High-risk clones have the propensity to disseminate rapidly, and genomics can improve the detection of their emergence and spread. This highlights the increasing need to build equitable partnerships to facilitate ownership transfer of genomic epidemiology capacity (operational, analytical and interpretational) to enhance national AMR surveillance programmes within low-resource settings. The binational partnership of the See and Sequence project led to a large retrospective survey of bacterial pathogens that has provided the first in-depth genomic view of the AMR landscape in the Philippines and established contextual data for ongoing local prospective sequencing, which commenced at the ARSRL in 2018. Through training and transfer of expertise in laboratory procedures and bioinformatics, the open exchange of data, and collective interpretation of results, we have expanded the existing capacity of a national reference laboratory with WGS focused on action for public health. While progress has been made, significant challenges remain to bring genomics into routine surveillance in low-resource settings. These include, and are not limited to, challenges in the supply chain and procurement of WGS reagents and equipment, vastly differing costs between high- and low-income settings, shortage of skilled local bioinformaticians due to both limited access to training and difficulties in staff retention, and the lack of platforms to feedback the actionable genomic data to sentinel sites. Nevertheless, we have generated a blueprint for the sustainable implementation of WGS and genomic epidemiology within national surveillance networks in LMICs which can be adapted and utilized within other locations to tackle the global challenge of AMR.

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Tables

Organism (code)	Key Resistance(s)	Antibiotics tested by ARSP	Genomes	Sentinel Sites
K. pneumoniae (Kpn)	Carbapenems, ESBL-producing	AMP, FOX, CAZ, CRO, FEP, IPM, AMC, TZP, GEN, AMK, CIP, SXT	340	18
E. coli (Eco)	Carbapenems, ESBL-producing	AMP, FOX, CAZ, CRO, FEP, IPM, AMC, TZP, GEN, AMK, CIP, SXT	181	17
P. aeruginosa (Pae)	Carbapenems	CAZ, FEP, IPM, MEM, TZP, GEN, TOB, AMK, CIP	176	17
A. baumannii (Aba)	Carbapenems	CAZ, CRO, IPM, SAM, TZP, GEN, AMK, CIP, SXT	108	13

Table 1. Organisms from the retrospective sequencing survey described in this study. The number of genomes sequenced, and the number of sentinel sites they represent are indicated. Key resistances were used to prioritize the isolates for WGS as described in the methods. Antibiotics tested by ARSRL for each organism are abbreviated according to their WHONET codes: AMC: amoxicillin/clavulanic acid, AMK: amikacin, AMP: ampicillin, CAZ: ceftazidime, CIP: ciprofloxacin, CRO: ceftriaxone, GEN: gentamicin, FEP: cefepime, FOX: cefoxitin, IPM: imipenem, MEM: meropenem, SAM: ampicillin/sulbactam, SXT: trimethoprim/sulfamethoxazole, TOB: tobramycin, TZP: piperacillin/tazobactam.

Plasmid	Size (bp)	Replicons	AMR genes	Match Accession (size)	Accession
p13ARS_MMH0112-2	243,650	IncFIB(K), IncFII(K)	blaCTX-M-15, blaTEM-1B, blaOXA-1, aac(3)-IIa, aac(6')-Ib-cr, aph(6)-Id, aph(3")-Ib, aph(3')-Ia, aac(6')-Ib-cr, qnrB1, mph(A), sul2, tet(A)	NA	ERS3609253
p13ARS_MMH0112-3	101,540	IncFII(Yp)	blaNDM-1, rmtC, sul1	MG462729.1 (110,787 bp)	ERS3609254

Table 2. Plasmids carrying carbapenemase genes and other resistance genes in *K. pneumoniae* ST340 strain 13ARS_MMH0112 from the Philippines. The replicons and AMR genes were identified with the plasmidFinder and Resfinder databases, respectively. Match Accession indicates the accession number when at least one match of more than 90% query sequence coverage and 99% identity was found in the NCBI nucleotide database.

Plasmid	Size (bp)	Replicons	AMR genes	Match Accession (size)	Accession
p14ARS_CVM0040-2	110,787	IncFII(Yp)	blaNDM-1, rmtC, sul1	MG462729.1 (110,787 bp)	ERS3609255
p13ARS_GMH0099	273,158	IncA/C2, IncR, IncFIA(HI1)	blaNDM-1, blaCTX-M-15, blaOXA-1, blaOXA-10, blaTEM-1B, aph(3')-VI, aph(3')-Ia, aadA1(x2), aac(6')-Ib-cr,aph(6)-Id, strA, strB, qnrS1, mph(A), erm(42), ere(A), floR, cmlA1(x2), ARR (x2), sul1(x3), sul2, dfrA14	NA	ERS3609256
p13ARS_VSM0593-1	181,414	IncA/C2	blaNDM-1, aph(3')-Ia, mph(A), cmlA1, floR, sul1, sul2, qnrS1, ARR-3,	NA	ERS3609257
p14ARS_MMH0055-5	44,885	IncX3	blaNDM-7	KP776609.1 (45,122 bp)	ERS3609258
p14ARS_VSM0843-1	95,284	IncR, IncFIA(HI1)	blaNDM-1, blaCTX-M-15, blaTEM-1B, aph(3')-VI, aph(6)-Id,aph(3")-Ib,aph(3')-VI, aadA1, cmlA1, sul1, sul2, ARR-3,	NA	ERS3609259

Table 3. Plasmids carrying NDM genes in *K. pneumoniae* ST147 representative isolates from the Philippines. The replicons and AMR genes were identified with the plasmidFinder and Resfinder databases, respectively. Match Accession indicates the accession number when at least one match of more than 90% query sequence coverage and 99% identity was found in the NCBI nucleotide database.

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Plasmid	Size (bp)	Replicons	AMR genes	Match Accession (size)	Accession
p14ARS_NMC0074-2	146,817	IncA/C2	blaNDM-1, blaOXA-10, aadA1, aph(3')-VI, aph(3')-Ia, mph(A), cmlA1, floR, sul1, sul2, dfrA14, qnrS1, ARR-2	NA	ERS3609260
p14ARS_NMC0074-5	51,479	IncX3	blaOXA-181, qnrS1	CP024806.1 (51,479 bp)	ERS3609263
p14ARS_NMC0074-4	81,712	IncFII/IncFIA	blaCTX-M-15, blaTEM-1B, blaOXA-1, aadA5, aac(6')-Ib-cr, sul1, tet(B), dfrA17	CP024805.1 (111,310 bp)	ERS3609262
p14ARS_NMC0074-3	82,418	IncFII	blaTEM-1, mph(A), erm(B)	NA	ERS3609261
p14ARS_NMC0074-6	2,088	Col(BS512)	-	CP024803.1 (2,088 bp)	ERS3609264

Table 4. **Plasmid repertoire of carbapenem-resistant *E. coli* strain 14ARS_NMC0074.** The replicons and AMR genes were identified with the plasmidFinder and Resfinder databases, respectively. Match Accession indicates the accession number when at least one match of more than 90% query sequence coverage and 99% identity was found in the NCBI nucleotide database.

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Figures

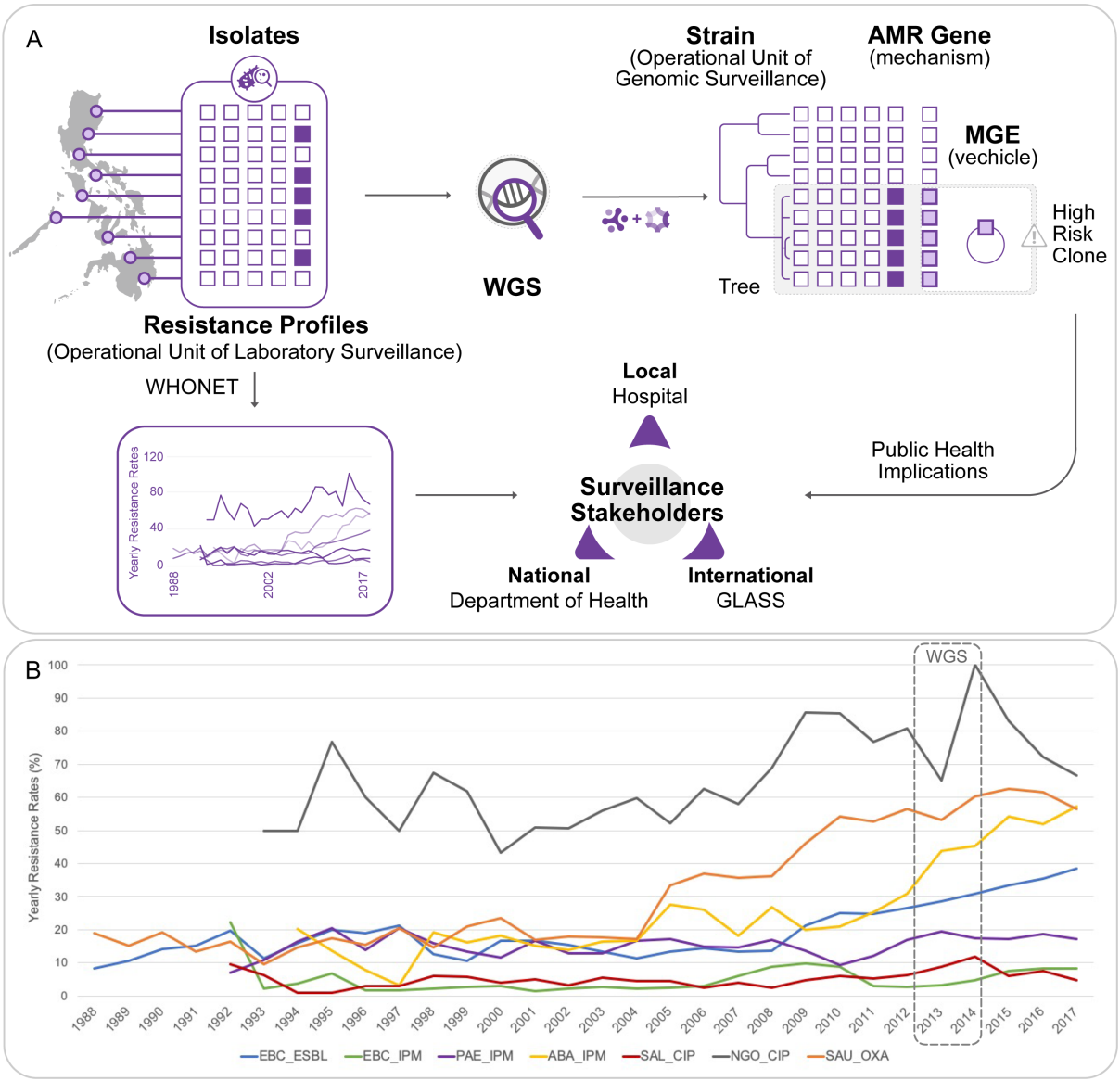

Figure 1. Implementing WGS for AMR surveillance in the Philippines. A) ARSP workflow and enhanced detection of high-risk clones by WGS. Isolates collected by sentinel sites are tested for susceptibility to antibiotics (open squares: susceptible, solid squares: resistant). The data are stored as resistance profiles in WHONET and summaries of resistance trends are shared yearly with surveillance stakeholders. Whole-genome sequencing (WGS) of bacterial isolates provides information on genetic relatedness (strains), known AMR mechanisms, and their vehicles for dissemination, allowing us to detect high-risk clones. **B)** Detail of trends in antimicrobial resistance in the Philippines. Yearly resistance rates for key bug-drug combinations based on phenotypic data collected by sentinel sites. EBC: Enterobacteriaceae (*K. pneumoniae*, *E. coli*, *Salmonella enterica*), PAE: *P. aeruginosa*, ABA: *A. baumannii*, but before 2000 *Acinetobacter* spp. SAL: *Salmonella enterica*, SAU: *S. aureus*. ESBL: Extended-spectrum beta-lactamase production suspected, or non-susceptible to the following antibiotics IPM: imipenem, CIP: ciprofloxacin, OXA: oxacillin. Dashed-rectangle labelled with WGS: period covered by the retrospective sequencing survey.

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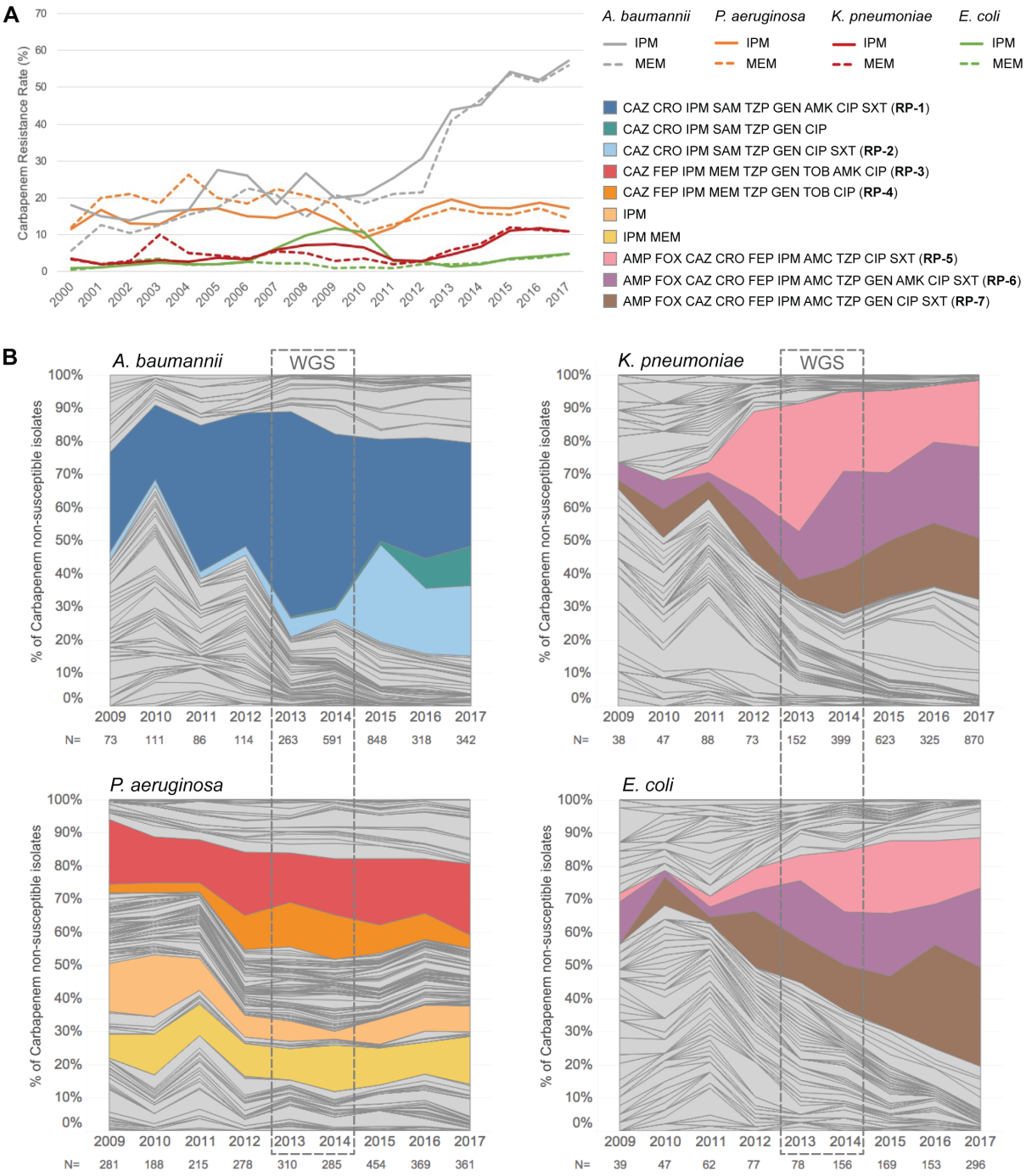

Figure 2. Temporal dynamics of the resistance profiles from carbapenem non-susceptible isolates. **A)** Yearly carbapenem resistance rates (IPM: imipenem, MEM: meropenem) for *P. aeruginosa*, *A. baumannii*, *E. coli* and *K. pneumoniae*. **B)** Relative abundance of resistance profiles with resistance to carbapenems (imipenem and/or meropenem). The three-letter code of an antibiotic indicates that the isolate is non-susceptible (resistant or intermediate) to the antibiotic. Only carbapenem non-susceptible isolates with complete susceptibility data were included, as indicated by the numbers under the the x-axis (N). Dashed-rectangle labelled with WGS: period covered by the retrospective sequencing survey.

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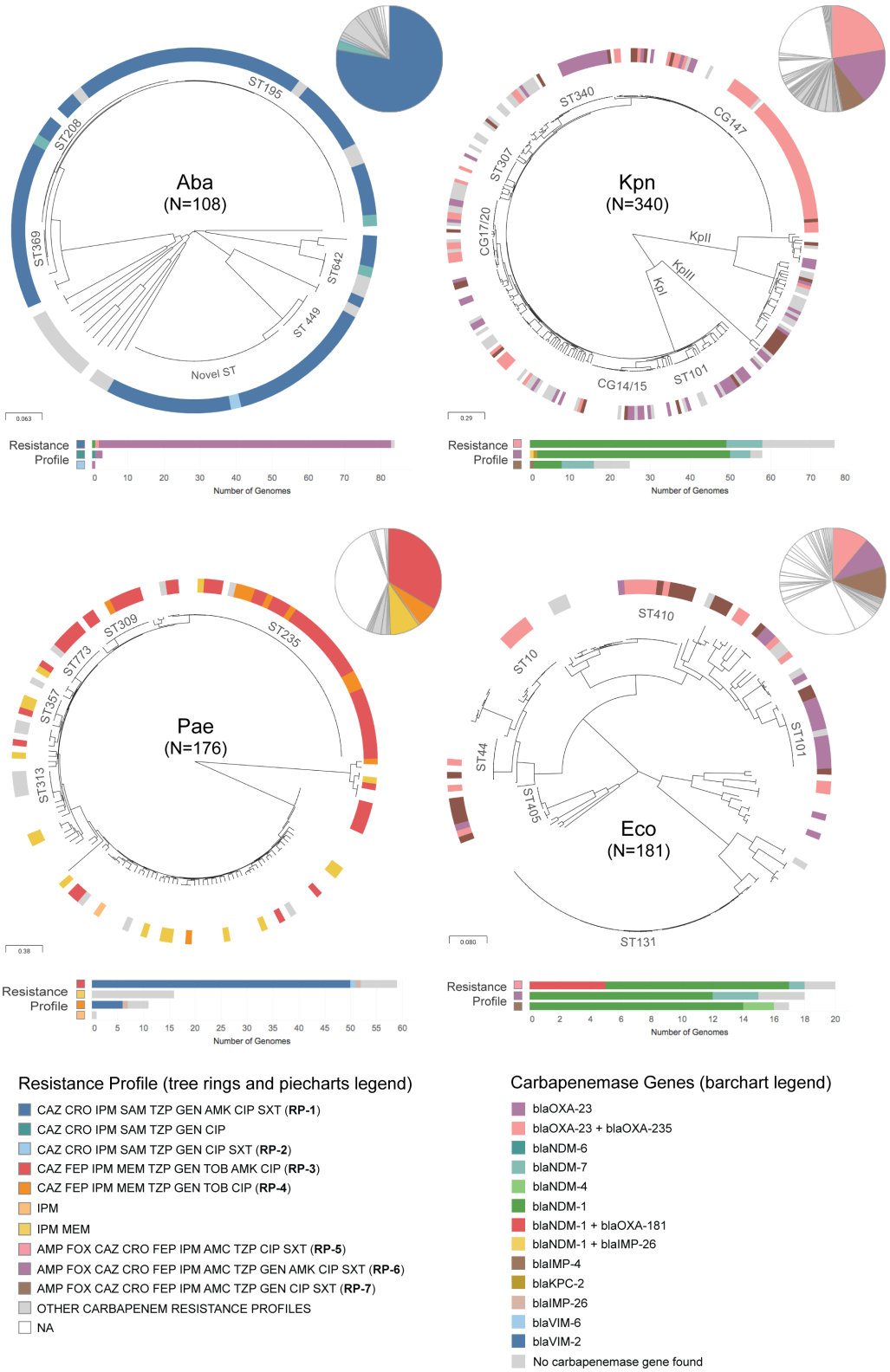

Figure 3. Resistance profiles are not associated with genetic lineages in carbapenem non-susceptible organisms. Phylogenetic trees showing the major lineages of *A. baumannii* (Aba), *P. aeruginosa* (Pae), *K. pneumoniae* species complex (Kpn), and *E. coli* (Eco), indicating the position of select STs and clonal groups (CGs). KpI: *K. pneumoniae sensu stricto*, KpII: *K. quasipneumoniae*, KpIII: *K. variicola*. Tree ring: Select carbapenem resistance profiles from Figure 2 are shown in colour. The remaining carbapenem resistance profiles are shown in light grey. Other profiles (ESBL and others) are omitted for simplicity (white). The pie charts show the relative abundance of the resistance profiles in the retrospective collection of sequenced genomes. The bar charts show the distribution of carbapenemase genes across the key resistance profiles. The data are available at https://microreact.org/project/ARSP_ABA_2013-14 (Aba), https://microreact.org/project/ARSP_PAE_2013-14 (Pae), https://microreact.org/project/ARSP_KPN_2013-14 (Kpn), and https://microreact.org/project/ARSP_ECO_2013-14 (Eco).

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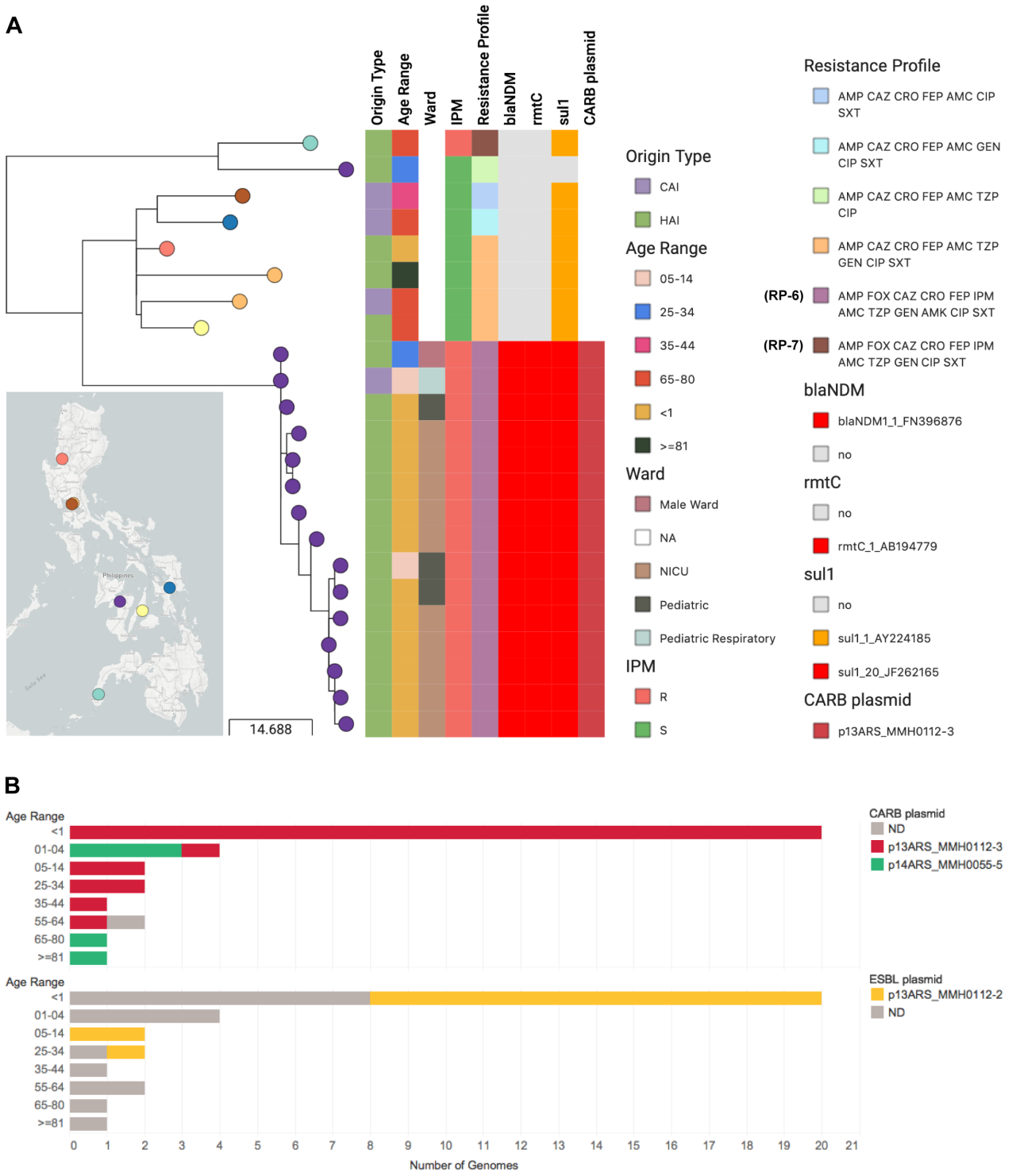

Figure 4. WGS reveals a previously undetected, plasmid-driven outbreak of *K. pneumoniae* ST340. A) Phylogenetic tree and linked epidemiological and genotypic data of 24 retrospective ST340 genomes. Maximum-likelihood tree inferred from 196 SNPs identified by mapping the genomes to reference CAV1217 (GCA_001908715), and masking regions of MGEs and recombination. The data is available at https://microreact.org/project/ARSP_KPN_ST340_2013-14. **B)** Distribution of 33 retrospective isolates from hospital MMH with resistance profile "AMP FOX CAZ CRO FEP IPM AMC TZP GEN AMK CIP SXT" by patient age group, with the distribution of STs (top panel) and plasmids with carbapenemases genes (bottom panel) indicated by the different colours. Short reads of the 33 isolates were mapped to the p13ARS_MMH0112-3 and the p14ARS_MMH0055-5 sequences and a plasmid match was counted when the reads covered at least 95% of the sequence length with at least 5x depth of coverage.

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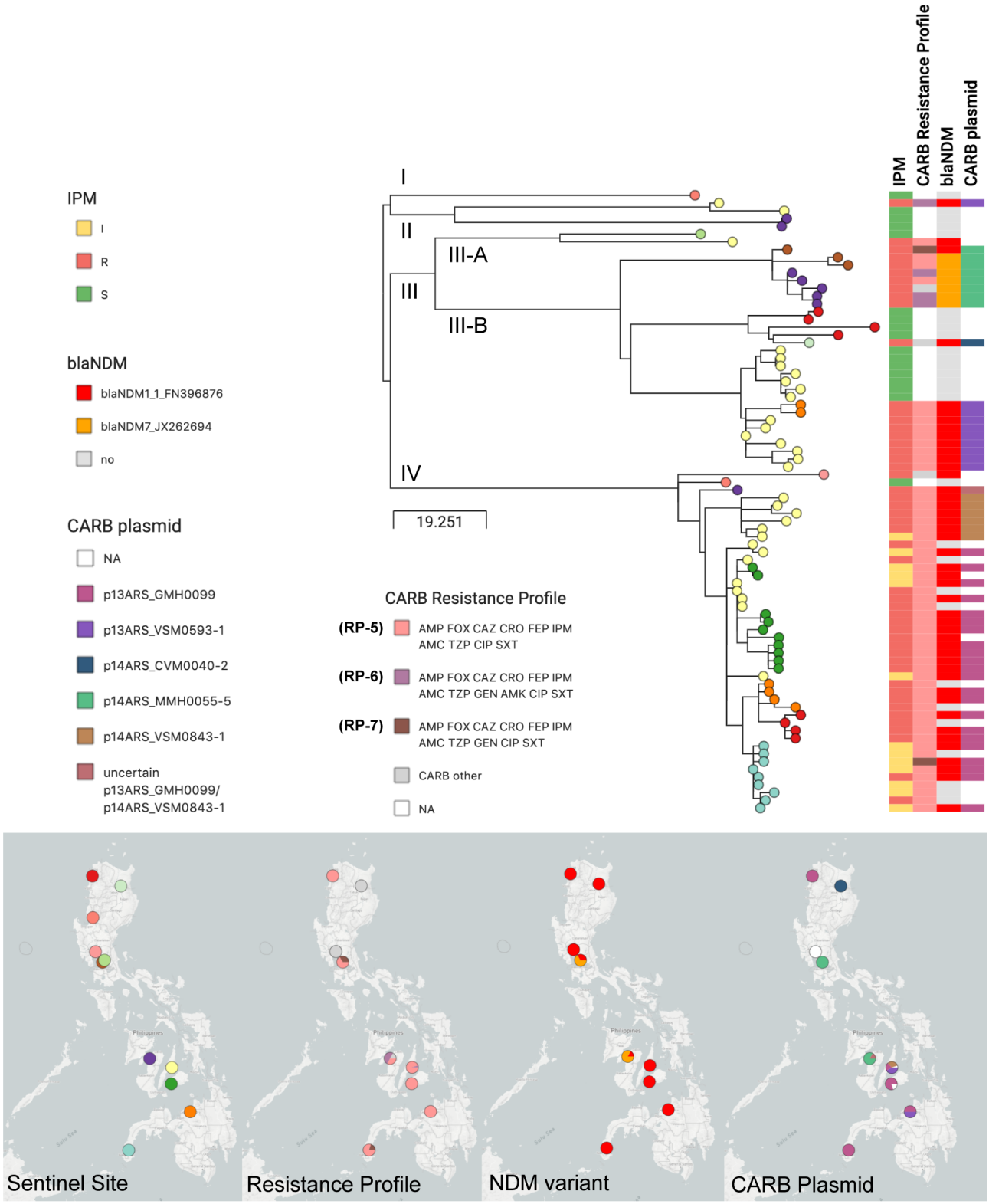

Figure 5. WGS reveals the circulation patterns of *K. pneumoniae* ST147 in the Philippines. Phylogenetic tree and linked epidemiological and genotypic data of 80 retrospective ST147 genomes. Maximum-likelihood tree inferred from 809 SNPs identified by mapping the genomes to reference MS6671 (LN824133.1), and masking regions of MGEs and recombination. The distribution of plasmids with carbapenemases genes was inferred by mapping the short reads of the genomes to the complete plasmid sequences, and a match was counted when the reads covered at least 95% of the sequence length with at least 5x depth of coverage. The data is available at https://microreact.org/project/ARSP_KPN_ST147_2013-14.

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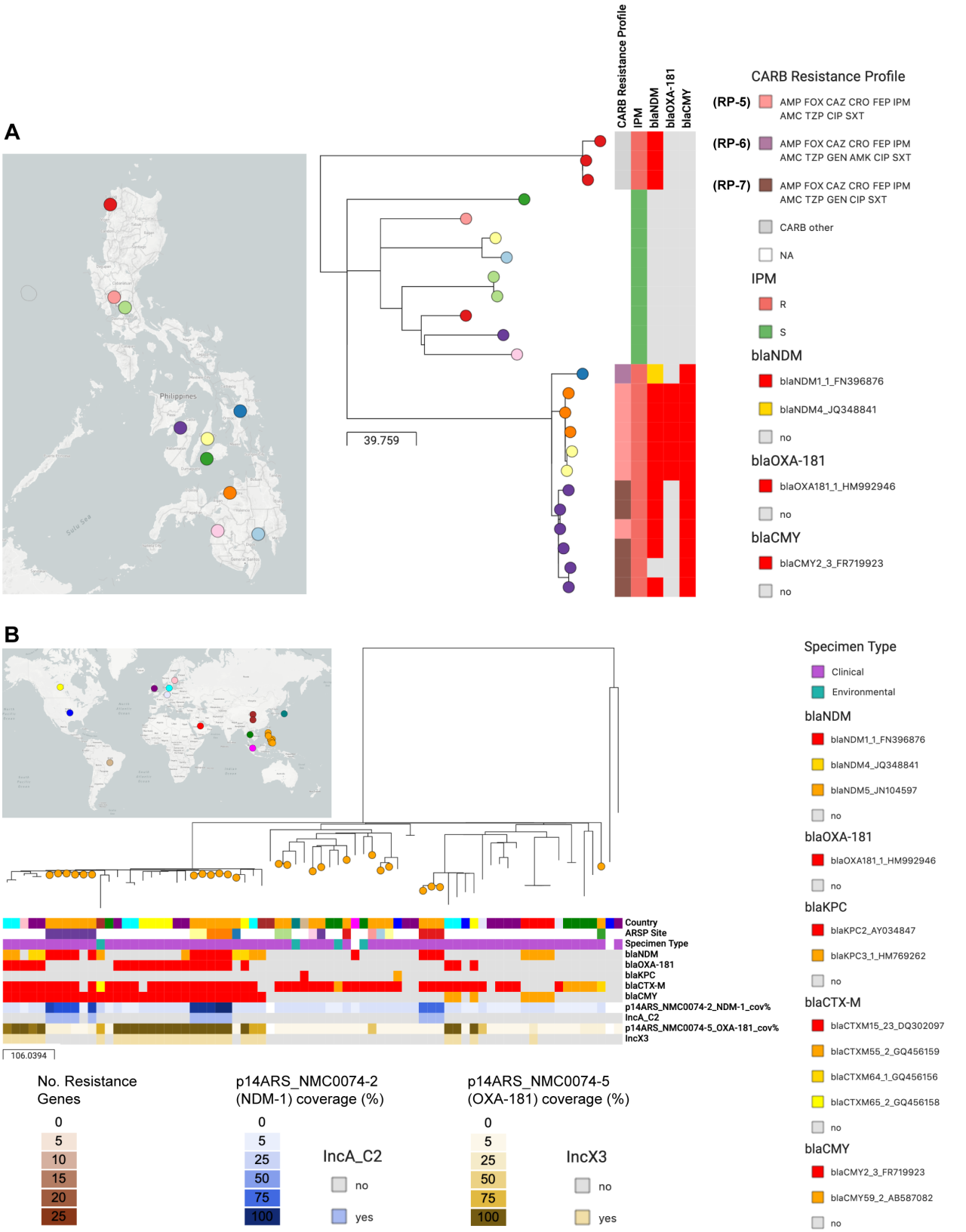

Figure 6. Phylogeographic analysis of *E. coli* ST410 from the Philippines. A) Phylogenetic tree and linked epidemiological and genotypic data of 24 retrospective ST410 genomes. **B)** Philippine isolates (orange nodes) in global context. Maximum-likelihood trees inferred from 703 (A) and 2851 (B) SNPs, respectively, identified by mapping the genomes to reference AMA1167 (CP024801.1), and masking regions of MGEs and recombination. The distribution of plasmids with carbapenemases genes in (B) was inferred by mapping the short reads of the genomes to the complete plasmid sequences, and a match was counted when the reads covered at least 95% of the sequence length with at least 5x depth of coverage. The data is available at https://microreact.org/project/ARSP_ECO_2013-14 (A) and https://microreact.org/project/ARSP_ECO_ST410_GLOBAL (B).