 Genome Sequencing Identifies Previously Unrecognized *Klebsiella pneumoniae* Outbreaks in Neonatal Intensive Care Units in the Philippines

Celia C. Carlos,1 Melissa Ana L. Masin,1 Marietta L. Lagrada,1 June M. Gayeta,1 Poile Krystle V. Macaranas,1 Sonia B. Sin,1 Maria Adelina M. Facun,1 J anziel Fiel C. Palarca,1 Aggettah M. Olorosa,1 Gicell Anne C. Cueno,2 Monica Abrudan,2 Khalil Abudahab,2 Silvia Argimón,2 Mihir Kekre,2 Anthony Underwood,2,3 John Stelling,3 and David M. Aanensen,2,4; for the NIHR Global Health Research Unit on Genomic Surveillance of Antimicrobial Resistance

1Research Institute for Tropical Medicine, Muntinlupa, the Philippines; 2Centre for Genomic Pathogen Surveillance, Wellcome Genome Campus, Hinxton, Cambridge, UK; 3Brigham and Women’s Hospital, Boston, MA, USA; and 4Centre for Genomic Pathogen Surveillance, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK.

**Background.** *Klebsiella pneumoniae* is a critically important pathogen in the Philippines. Isolates are commonly resistant to at least 2 classes of antibiotics, yet mechanisms and spread of its resistance are not well studied.

**Methods.** A retrospective sequencing survey was performed on carbapenem-, extended spectrum beta-lactam-, and cephalosporin-resistant *Klebsiella pneumoniae* isolated at 20 antimicrobial resistance (AMR) surveillance sentinel sites from 2015 through 2017. We characterized 259 isolates using biochemical methods, antimicrobial susceptibility testing, and whole-genome sequencing (WGS). Known AMR mechanisms were identified. Potential outbreaks were investigated by detecting clusters from epidemiologic, phenotypic, and genome-derived data.

**Results.** Prevalent AMR mechanisms detected include *blaCTX-M-15* (76.8%) and *blaNDM-1* (37.5%). An epidemic IncFII(Yp) plasmid carrying *blaNDM-1* was also detected in 46 isolates from 6 sentinel sites and 14 different sequence types (STs). This plasmid was also identified as the main vehicle of carbapenem resistance in 2 previously unrecognized local outbreaks of ST348 and ST283 at 2 different sentinel sites. A third local outbreak of ST397 was also identified but without the IncFII(Yp) plasmid. Isolates in each outbreak site showed identical STs and K- and O-loci, and similar resistance profiles and AMR genes. All outbreak isolates were collected from blood of children aged < 1 year.

**Conclusion.** WGS provided a better understanding of the epidemiology of multidrug resistant *Klebsiella* in the Philippines, which was not possible with only phenotypic and epidemiologic data. The identification of 3 previously unrecognized *Klebsiella* outbreaks highlights the utility of WGS in outbreak detection, as well as its importance in public health and in implementing infection control programs.

**Keywords.** antimicrobial resistance; *K. pneumoniae*; outbreak detection; whole genome sequencing.

Antimicrobial resistance (AMR) is a serious threat to public health because antimicrobial-resistant pathogens limit therapeutic options and result in increased morbidity and mortality [1]. AMR is also perceived as a threat to the achievement of the Sustainable Development Goals [2].

AMR surveillance has conventionally been performed by monitoring distribution of antimicrobial-resistant pathogens in the population through phenotypic methods such as antimicrobial susceptibility testing, standard culture, and bacterial serotyping for identification and characterization [3, 4]. WHONET is also widely used for surveillance data collection and analysis, whereas SaTScan integrated with WHONET allows early and broad detection of event clusters using retrospective or prospective algorithms and other flexible spatial and/or temporal scan parameters [5-7].

In the Philippines, priorities and methods for surveillance of AMR, hospital-acquired and community-acquired infections are determined by the local healthcare facility’s infection control committee in coordination with the microbiology laboratory [8]. Hospital bacterial isolates, antibiograms, and clustering of patient groups within the hospital network are monitored and reviewed in a set time frame, and semiannual infection rates and antibiograms are reported to clinicians and administrators [8]. Suspected outbreaks or the occurrence of uncharacteristically large numbers of cases are reported to the local office of the National Epidemiology Center—Department of Health for appropriate action [8, 9].
Bacterial typing based on phenotypes, however, fails to distinguish isolates that have the same resistance profiles (RPs) and isolates belonging to closely related *Klebsiella* species [3, 10, 11]. The development of molecular methods such as pulsed-field gel electrophoresis and multilocus sequence typing (MLST) enabled molecular detection of relatedness of isolates, but these are labor-intensive, time-consuming, expensive, and often of a low resolution insufficient for outbreak analysis because they assay variation at small proportions of the genome [12]. The decreasing costs of whole-genome sequencing (WGS) can address these limitations by providing high-resolution subtypes of AMR pathogens [13, 14] and identifying AMR genes and their location on bacterial chromosomes or on plasmids [15, 16]. Through genome-wide analysis, a more granular picture of the status of AMR can potentially be determined by demonstrating mechanisms of AMR, transmission of AMR genes, and relatedness of strains [14-16].

*Klebsiella pneumoniae* is considered a microorganism of public health importance in the Philippines and is classified as critically important by the World Health Organization [17]. It is one of the leading causes of hospital-acquired infections, especially among the immunocompromised [18, 19]. Carbapenem-resistant *K. pneumoniae* was first isolated in the Philippines between 1992 and 1994 and has been molecularly characterized in recent years [15, 20]. A local outbreak of carbapenem-resistant ST340 and its possible transmission through an IncFII(Yp) plasmid with *bla*<sub>NDM-1</sub>, *rmtC*, and *sul1* was also identified through retrospective WGS survey, leading to the hospital's review of infection control protocols and implementation of more stringent programs [15].

In this study, a retrospective sequencing survey was undertaken on carbapenem-resistant, cephalosporin-resistant, and/or extended spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* isolated from 2015 to 2017 to provide genomic context for local prospective surveillance. WGS analysis identified 3 potential local outbreaks among neonates, which were confirmed by corroborating epidemiological information, such as close isolation times and overlapping locations.

**METHODS**

**Bacterial Isolates**

From 2295 ESBL-positive, and/or cephalosporin resistant, and/or carbapenem-resistant *Klebsiella* isolates referred to the Antimicrobial Resistance Surveillance Reference Laboratory in 2015–2017, 263 (11.5%) were selected for WGS based on the following criteria: with complete RPs or at most 2 not tested antibiotic; overall prevalence of RPs (including both referred and unreviewed isolates); geographical representation of sentinel sites; prioritized invasive isolates when both invasive and noninvasive isolates representing a combination of RPs, sentinel site, and collection year were available (Supplementary Methods) [15]. The isolates were retrieved and resuscitated on Tryptic Soy Broth and incubated overnight at 35°C for reidentification and retesting of key phenotypic resistance (Supplementary Methods).

**DNA Extraction and WGS**

Genomic DNA was isolated using nexttec 1-Step DNA Isolation Kit for Bacteria (nexttec Biotechnologie GmbH, 20N. 904) in accordance with the manufacturer's instructions. DNA was quantified using Quantifluor dsDNA System (Promega, E2670) and Quantus Fluorometer (Promega, E6150), and then sent to the Wellcome Trust Sanger Institute for sequencing using Illumina HiSeq2500 platform with 100- or 250-base paired-end reads. A total of 259 (98.5%) isolates passed quality control and were included in the study. Raw sequence data generated were deposited in the European Nucleotide Archive under the project accession PRJEB29738. Run accessions are provided in Microreact projects linked in the figure descriptions [21].

**Bioinformatics Analysis**

The following analyses were performed using pipelines developed within the National Institute for Health Research Global Health Research Unit on Genomic Surveillance of AMR: quality control, de novo assembly, mapping-based single nucleotide polymorphism (SNP) phylogeny, AMR, and MLST predictions [22-29]. Briefly, the sequences were assembled using SPAdes and identified using BactInspector; contamination was detected using confindr [30-32]. Fastqc, multiqc, and qualiyfr were used for quality control [27-29]. SNP-based phylogeny was generated by mapping reads to a reference sequence using BWA mem; variants were called and filtered using bcftools, and a maximum likelihood phylogeny was produced using IQTree and reference genome *K. pneumoniae* strain K2044 (GCA_009497695.1) [33-35].

Pathogenwatch was used to identify MLST, K- and O-loci, virulence factors, and plasmid replicons [36]. AMR genes were predicted using ARIBA 2.14.4 in conjunction with the NCBI AMR acquired gene and PointFinder databases [37, 38]. Sequences were analyzed using PlasmidFinder and mapped against *bla*<sub>NDM</sub> plasmids p13ARS_MMH0112-3 and p14ARS_MMH0055-5 [39]. Only those with ≥ 95% coverage were considered as matched. Results were collated and uploaded to Microreact for visualization [21].

**Outbreak Analysis**

Isolates with identical locations, forming clusters in the phylogenetic tree, were inspected as potential outbreaks. Maximum-likelihood phylogenetic trees were generated for each cluster using reference genomes EuSCAPE_IL028, EuSCAPE_DK005, and SRR5514218. Epidemiologic data, antimicrobial susceptibility testing results, and genotypic characteristics of isolates in each cluster were then investigated. Infection origin was...
computed based on date of admission and sample collection date. Positive isolates collected more than 2 days after hospital admission were determined to be hospital acquired. Meanwhile, positive isolates collected 0–2 days before hospital admission were classified as community acquired.

WHONET-SaTScan’s space-time scan permutation simulated prospective was used to look for statistical clusters among all isolates (including unreferred isolates) recovered from the outbreak sites in 2015–2017 and characterized by the same RPs as the outbreak isolates [7]. SaTScan analysis was also extended to 2019 to check the persistence of the RP at the sentinel site. A maximum cluster length of 365 days and a recurrence interval (RI) of > 365 days were set to exclude random signals that occur by chance alone and are of limited epidemiologic significance [40].

RESULTS
Isolate Distribution and Characteristics
The 259 Klebsiella isolates were collected between 2015 and 2017 by 20 of 26 Antimicrobial Resistance Surveillance Program sentinel sites representing 16 of 17 regions (Supplementary Table 1). Isolates were submitted as carbapenem-resistant (n = 81, 31.2%), carbapenem- and cephalosporin-resistant (n = 58, 22.4%), cephalosporin-resistant (n = 11, 4.2%), ESBL-producing (n = 62, 23.9%), and ESBL-producing and cephalosporin-resistant (n = 47, 18.1%) (Supplementary Table 2).

Invasive isolates from blood (n = 240, 92.7%) and cerebrospinal fluid (n = 13, 5.0%) were prioritized for WGS. A few noninvasive samples (ie, urine [n = 3], sputum [n = 1], tracheal aspirate [n = 1], and umbilical cord [n = 1]) were also analyzed based on their resistance profiles.

The majority of the isolates were from inpatients (n = 253, 97.7%) and from hospital-acquired infections (n = 171, 66.0%). Isolates were collected from patients aged < 1 to 93 years old, but most were from patients < 1 year old (n = 145, 56.0%), composed of 84.8% neonates (0–28 days) and 15.2% infants (29 days–11 months). Hence, infections were mostly detected in the neonatal department (n = 77, 29.7%). Other patients aged < 1 year were also admitted to the intensive care unit (ICU), pediatric, pediatric ICU, mixed ward, and emergency departments.

Species Identification and Sequence Type
In silico species identification resulted in 214 (82.6%) K. pneumoniae, 36 (13.9%) Klebsiella quasipneumoniae subsp.

Figure 1. Phylogenetic tree of 259 Klebsiella isolates showing deep branches separating Kp I (K. pneumoniae) and Kp II (K. quasipneumoniae). Clusters based on linked genotypic (ST, KL, and O locus types) data showed 3 clusters of possible NICU outbreaks in 3 separate hospitals. Most ST348 isolates were collected in CMC, whereas ST397 and ST283 were unique in VSM and JLM, respectively. Maximum-likelihood tree was inferred from mapping genomes to reference K. pneumoniae strain K2044 (GCA_009497695.1). This interactive view is available at: https://microreact.org/project/pBoycZe8jwz3Aghc3EE99bc. NICU, neonatal intensive care unit.
**similipneumoniae**, and 9 (3.5%) *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* (Figure 1). The 45 *K. quasipneumoniae* were also correlated with 45 identified *bla*<sub>RXP</sub> genes, a *K. quasipneumoniae* chromosomal marker encoding ampicillin resistance [11]. The overlapping biochemical phenotypes and lack of a stable classifier among these closely related species may account for the inability of conventional laboratory techniques to definitively differentiate them [11, 41].

There were 102 different MLSTs predicted using ARIBA (Supplementary Figure 1). The most common were sequence type (ST)147 (n = 23, 8.9%), ST348 (n = 19, 7.3%), ST14 (n = 10, 3.9%), and ST283 (n = 9, 3.5%) (Supplementary Table 3). Ten isolates were identified to have novel ST profiles.

**Capsular and Lipopolysaccharide Typing**

The *K. pneumoniae* capsule has been shown to be a key virulence determinant, suppressing host inflammatory response and providing resistance to antimicrobial peptides [42-44]. Capsular and lipopolysaccharide typing using the K and O loci identified 57 different K loci (KL) with good or higher confidence for 244 isolates, and 14 O loci for 256 isolates (Supplementary Figure 2). The most prevalent KL types were KL62 (n = 22, 8.5%), KL10 (n = 20, 7.7%), and KL64 (n = 16, 6.2%). The most prevalent O loci were O1v1 (n = 69, 26.6%), O1v2 (n = 34, 13.1%), and O5 (n = 31, 12.0%).

**Resistance Profiles and AMR Genes**

A total of 93 RPs were observed, with the 4 most common accounting for 32.3% of the isolates (Supplementary Figure 1). RP-1, RP-3, and RP-4 were extremely drug-resistant (XDR) RPs of *K. pneumoniae* and *Escherichia coli* reported to be expanding in the Philippines [15].

Known AMR mechanisms for carbapenem resistance, cephalosporin resistance, and ESBLs were identified (Supplementary Table 2). The most prevalent were the ESBL *bla<sub>CTX-M-15</sub>* gene (n = 199, 76.8%) and the carbapenemase *bla<sub>NDM-1</sub>* gene (n = 97, 37.5%). Other carbapenemase genes included *bla<sub>NDM-2</sub>* (n = 18, 6.9%), *bla<sub>KPC-2</sub>* (n = 4, 1.5%), and *bla<sub>NDM-9</sub>* (n = 1, 0.4%). Nineteen isolates had unknown mechanisms but carried combinations of *bla* genes and *ompK36* and *ompK37* mutations, which have been linked to carbapenem resistance in *Enterobacteriaceae* [45].

*rmtC*, encoding high resistance to aminoglycosides, was also identified in 48 (18.9%) isolates [46]. Other prevalent aminoglycoside resistance genes were: *aac(6’)-30/aac(6’)-Ib’* (81.5%), *aac(6’)-Ib* (81.5%), *aac(3’)-II* (46.3%), *aph(3’’)-Ib* (47.9%), and *aph(6)-Id* (47.9%).

Fluoroquinolone resistance genes *oqxA* (95.4%) and *oqxB* (95.4%) were also observed in most isolates, along with *qnrB1* (32.8%), *qnrS1* (33.2%), *qnrB6* (12.7%), and *qnrB4* (4.2%). However, these genes only confer low-level resistance or reduced susceptibility, which may not necessarily translate to phenotypic resistance [43]. Single *gyrA* mutations at codon 83 (11.2%) and double mutations at codon 83 and 87 (9.3%) were also found in some isolates. A single par*C* mutation cooccurred with 52 of 53 *gyrA* mutations (98.1%). The *gyrA* and *par*C mutations corresponded to ciprofloxacin resistance in 50 of 53 isolates (94.3%). Sulfonamide and trimethoprim resistance genes *dfrA* (88.4%), *sul1* (70.7%), and *sul2* (58.3%) were also detected.

**Inc Type Profiling**

A total of 32 different Inc types were observed among the 259 isolates (Supplementary Figure 1). IncFIB(K) (n = 201, 21.4%) and IncFIII(K) (n = 163, 17.3%) were the most prevalent. Notable Inc type profiles were IncFIII(Yp)-*bla<sub>NDM-1</sub>*-*rmtC*, and *sul1* (p13ARS_MMH0112-3) was previously linked to a nosocomial outbreak of *K. pneumoniae* ST340 in the Philippines [15]. The same IncFIII(Yp) and AMR genes were also present in 46 of 48 IncFIII(Yp)-positive isolates from 6 sentinel sites and 14 different STs (Supplementary Table 4). Short reads of all 46 isolates and 1 IncFIII(Yp)-*bla<sub>NDM-2</sub>* mapped to the plasmid with > 95.0% coverage of the sequence length. There were 21 IncX3 replications found in the genomes of 19 of 20 *bla<sub>NDM-7</sub>*-carrying isolates from 9 sentinel sites and 8 STs (Supplementary Table 4). Short reads of all 19 were mapped to the *bla<sub>NDM-2</sub>*-carrying plasmid p14ARS_MMH0055-5 with 100% coverage of the plasmid sequence [15]. These results suggest that both IncFIII(Yp) and IncX3 plasmids have been widely circulating and conferring carbapenemase resistance to a diverse genetic background, including non-epidemic strains, in the Philippines [15]. This study is limited by short-read data, hence further plasmid or long-read sequencing will be conducted to characterize other vehicles of AMR gene transmission.

**Local Outbreak of *K. pneumoniae* ST348**

WGS paired with epidemiological data provided a phylogenetic tree with 3 observed clusters (CMC, VSM, JLM) in 3 separate hospitals (Figure 1). This prompted an investigation to identify possible disease outbreaks. In all 3 clusters, all isolates were collected from blood and affected patients were all aged < 1 year. Isolates in each cluster had identical MLST, capsular, and lipopolysaccharide types, and similar RPs and AMR genes (Figure 2). In addition, all the isolates in the CMC and JLM clusters carried plasmid p13ARS_MMH0112-3 (Supplementary Figure 3).

The largest cluster was observed in CMC, with 15 isolates collected between September 2016 and August 2017 identified as ST348 (Figure 2A). Most of the cases were hospital acquired (n = 11, 73.3%) with admissions to the neonatal ward (66.7%), pediatric ward (20.0%), and ICU (13.3%). All isolates had the same K L2 and O1v1 loci and carried virulence factor *yersiniabactin* (*ybt14*). Mean pairwise SNP differences between these 15 isolates was 6.3 SNPs (range: 1–28), suggesting intrahospital transmission when compared with the 53.1 mean pairwise SNP differences (range: 1–236) of other ST348
There were 14 ST348 isolates (93.3%) exhibiting RP-2, whereas 1 (6.7%) was additionally resistant to CIP exhibiting RP-1, which was confirmed by retesting the isolate (Figure 2A). Because gyrA and parC mutations were absent, this might have been caused by unknown mechanisms or differences in gene expression despite having similar low-level fluoroquinolone resistance genes, such as oqxAB or aac(6')-Ib-cr [49].

Local Outbreak of K. pneumoniae ST397
The second cluster was identified at sentinel site VSM, with 7 isolates collected from April 1 to May 2, 2016, identified as ST397. All patients were neonates, and cases were determined to be hospital acquired (Figure 2B). KL158 and O1v1 loci and yersiniabactin gene ybt9 were also identified in all isolates. The mean pairwise SNP difference between isolates was 3.9 SNPs (range: 1–8), indicating origin from a single transmission cluster [47, 48]. ST397 genomes from other hospitals were not available for comparison.
All isolates were ESBL producing, with 57.1% (n = 4) exhibiting RP-5 (full profile is shown in Figure 2), corresponding to isolates carrying IncFIA(pBK30683). Loss of IncFIA(pBK30683) in 3 isolates was also concordant with observed loss of aac, blα(6′)-Ib-cr, cat, dfrA, and qnrB1, indicating that these genes may be carried on an IncFIA(pBK30683) plasmid.

Local Outbreak of K. quasipneumoniae ST283
The third cluster from JLM comprised 9 isolates collected from neonates in May 2016–July 2017. Most cases were determined to be hospital acquired (66.7%) (Figure 2C). All isolates were previously identified as K. pneumoniae by biochemical methods, but as K. quasipneumoniae subsp. similipneumoniae ST283 by WGS. All had the same KL10 and O5 loci. The mean pairwise SNP difference between isolates was 3.75 SNPs (range: 0–10), suggesting intrahospital outbreak [47, 48]. ST283 genomes from other hospitals were not available for comparison.

Eight isolates (88.9%) exhibited RP-1, whereas 1 (11.1%) additionally showed intermediate susceptibility to CIP (RP-2), which was confirmed by retesting the isolates. This is possibly due to unknown mechanisms or differences in gene expression, despite having the same aac(6′)-Ib-cr, qoxAB, and qnrB1 genes conferring low-level fluoroquinolone resistance because gyrA and parC mutations were not detected [49].

WHONET-SaTScan Analysis
WHONET-SaTScan analysis of CMC Klebsiella isolates from 2015 through 2019 detected a statistically significant cluster for RP-2 (P < .0013, RI = 789 days) (Table 1), which occurred in July 2017. This overlapped with the WGS-identified outbreak period of September 2016–August 2017. Within this RP-2 cluster (n = 10), we detected 3 of 4 ST37 isolates that mapped to the IncFII(Yp) plasmid (Supplementary Figure 3), and only 4 of 14 WGS-identified ST348 outbreak isolates (28.6%). Extending the analysis to 2019 also showed an RP-8 cluster (n = 3) occurring in December 2018, indicating the possible persistence of AMR in VSM.

Last, WHONET-SaTScan analysis of all JLM Klebsiella isolates (n = 1562) generated no clusters for either outbreak RP, although RP-1 and RP-2 have been observed in 12 and 29 cases, respectively, from 2015 through 2019 (Supplementary Figure 4). This suggests an even distribution or a gradual increase of cases, rather than a sudden increase, which is indicative of an outbreak signal detected by SaTScan. Altogether, results showed that cluster analysis with WHONET-SaTScan and a fixed set of parameters may not detect all clusters, especially if they comprise few isolates exhibiting more than 1 RP. Scan type and spatial and/or temporal parameters may, however, be refined to detect clusters not only among RPs but also in specific wards.

**DISCUSSION**
We undertook a retrospective WGS survey of K. pneumoniae covering the years 2015–2017. We identified 3 outbreaks of Klebsiella among neonates in different hospitals in the Philippines, based on clusters observed in the phylogenetic tree, which resulted from combined epidemiologic and genotypic information. Average SNP differences in the 3 outbreaks were lower than the suggested thresholds of 16 and 21 SNPs for a K. pneumoniae intrahospital outbreak [47, 48].

Of the 3 outbreak strains, ST397 and ST283 were unique to VSM and JLM, respectively, but were nevertheless identified in other countries [50-53]. However, the same STs in other

**Table 1. Clusters Detected by WHONET-SaTScan Analysis of CMC and VSM Klebsiella isolates, 2015–2019**

| Resistance profile no. | Resistance profile | Recurrence interval | Cluster P value | Cluster date | No. observed | No. of outbreak isolates in cluster |
|-----------------------|-------------------|---------------------|----------------|--------------|-------------|-----------------------------------|
| CMC                   |                   |                     |                |              |             |                                   |
| 1                     | AMP FOX CAZ CRO FEP IPM AMC TZP GEN AMK CIP SXT | 1008 | .000992 | July–August 2018 | 4 | None |
| 2                     | AMP FOX CAZ CRO FEP IPM AMC TZP GEN AMK CIP SXT | 789 | .00127 | July 2017 | 10 | 4 |
| VSM                   |                   |                     |                |              |             |                                   |
| 5                     | AMP CAZ CRO AMC GEN AMK CIP | 10 369 | .000964 | April 2016 | 4 | 4 |
| 6                     | AMP CAZ CRO AMC GEN AMK CIP | 4575 | .000219 | March–April 2016 | 7 | 1 |
| 7                     | AMP CAZ CRO       | ...                 | ...            |             |            |                                   |
| 8                     | AMP CRO           | 1220 | .00082 | December 2018 | 3 | None |
countries had differing AMR gene complements compared with the Philippine outbreak strains, which may therefore relate to local antimicrobial use practices [54].

For some isolates of the same outbreak strain, the complement of AMR genes differed among isolates of the same lineage, indicating distinct and potentially quite frequent gene-acquisition events, especially among hospital isolates. It is postulated that these AMR genes are acquired through selection because of antimicrobial exposure during hospital stay and may be carried by possibly epidemic plasmids circulating among nonepidemic strains, such as p13ARS_MMM0112-3, 1 of the main drivers of the JLM and CMC outbreaks [54]. Its occurrence in the genetic background of multiple STs within nonepidemic clones and at multiple sentinel sites suggests this is an epidemic plasmid that causes outbreaks among the immunocompromised, such as neonates in the ICU. However, further plasmid studies are needed to identify and characterize more AMR vehicles and modes of transmission.

There were no identified hypervirulent, extremely resistant K. pneumoniae, such as the epidemic KPC-producing ST258/ST11 clonal complex (CC258). However, possible XDR RPs (RP-1, RP-3, RP-4) (n = 63, 24.2%) were among the most observed in this study, suggesting a possibly ongoing expansion [15]. Because surveillance of AMR phenotypes is monitored, it may also be worthwhile to include surveillance of high-risk clones such as CC258 to predict invasive disease [54].

Using WGS, we were also able to distinguish what was phenotypically identified as K. pneumoniae to be K. quasipneumoniae. The phylogenetic tree of the 259 genomes in the Philippines showed deep branches separating the K. pneumoniae complex into K. pneumoniae and K. quasipneumoniae. K. quasipneumoniae is said to be less pathogenic than K. pneumoniae, which is more frequently associated with colonization or hospital-acquired infections [55].

The WGS pipeline was more efficient than WHONET-SaTScan at identifying potential outbreaks because it was able to recognize the JLM outbreak, which SaTScan failed to do using RP as the lone parameter. However, WHONET-SaTScan may still be a good complement for WGS as demonstrated in CMC because it was able to tag nonoutbreak strains carrying the same epidemic plasmid as the confirmed outbreak isolates. Clinical interpretation is still based on the insights of clinical and infection control staff [7]. Further, the method may be limited by hospital policies governing the choice of antibiotics for testing and reporting of results, which impacts on the configuration of WHONET [56]. On the other hand, sequence data can provide a broader range of genotype information about isolates, which allows better characterization through improved molecular resolution. To maximize the use of genomics in identifying outbreaks and to inform infection control programs, it may also be beneficial to sequence isolates from clusters identified by WHONET-SaTScan.

Review of resistance profiles in the 3 hospitals showed persistence of the possible XDR RPs in CMC and JLM, indicating that more aggressive infection control interventions may be necessary to control the continuing expansion. We have communicated with the infection control staff of both hospitals to alert them, and they have implemented aggressive measures to prevent future outbreaks. These outbreaks illustrate that the routine utility of both WHONET-SaTScan and WGS in context of retrospective data will enable real-time generation of alerts, early outbreak detection and investigation, and immediate infection control [57].

In conclusion, WGS was more specific and sensitive in identifying antimicrobial resistant strains than our current epidemiologic and phenotypic methods of surveillance. It provided a more in-depth understanding of AMR epidemiology of multidrug-resistant Klebsiella in the Philippines. This resulted in the identification of 3 previously unrecognized local outbreaks of K. pneumoniae and K. quasipneumoniae among neonates in 3 distinct areas, which was not possible using phenotypic data alone. Sustaining WGS can improve public health services to identify patients with distinct AMR sequences who are at risk of treatment failure, to predict potential outbreaks, and to take action for their immediate control.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

**Acknowledgments.** Members of the NIHR Global Health Research Unit on Genomic Surveillance of Antimicrobial Resistance: Harry Harste, Dawn Muddyman, Ben Taylor, Nicole Wheeler, and Sophia David of the Centre for Genomic Pathogen Surveillance, Big Data Institute, University of Oxford. Old Road Campus, Oxford, UK, and Wellcome Genome Campus, Hinxton, UK; Pilar Donado-Godoy, Johan Fabian Bernal, Alejandra Arevalo, Maria Fernanda Valencia, and Erik C. D. Osma Castro of the Colombian Integrated Program for Antimicrobial Resistance Surveillance—Coipars, CI Tibaíta, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Tibaíta—Mosquera, Cundinamarca, Colombia; K. L. Ravikumar, Geetha Nagaraj, Varun Shamanna, Vandana Govindan, Akshata Prabhu, D. Sravani, M. R. Shincy, Steffimole Rose, and Ravishankar K.N of the Central Research Laboratory, Kempegowda Institute of Medical Sciences, Bengaluru, India; Iruka N. Okeke, Anderson O. Oaikhen, Ayorinde O. Afolayan, Jolaade J. Ajiboye, and Erikson Ewomazino Odih of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria; Ali Molloy, alimolloy.com; and Carolin Vegvari, Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, India; Iruka N. Okeke, Anderson O. Oaikhen , Ayorinde O. Afolayan, Jolaade J. Ajiboye, and Erikson Ewomazino Odih of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria; Ali Molloy, alimolloy.com; and Carolin Vegvari, Imperial College London.

We are grateful to the members of the Antimicrobial Resistance Surveillance Program (Supplementary Note) that collected bacterial isolates and linked epidemiological data, especially to sentinel sites CMC, VSM, and JLM for cooperating and for providing additional information. We are also grateful to our project and support staff Elmer M. Herrera Jr., Laila T. Flores, Karis Lee D. Boehme, and Michael F. Domingo, and our consultant Dr. Charmian M. Hufano for all their assistance.
**Supplement sponsorship.** The supplement is supported by the UK National Institute for Health Research Global Health Research Unit on Genomic Surveillance of Antimicrobial Resistance (GHRU).**

**Financial support.** This work was supported by Official Development Assistance (ODA) funding from the National Institute for Health Research (Wellcome Trust grant number 206194).

This research was commissioned by the National Institute for Health Research using Official Development Assistance (ODA) funding. Views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research, or the Department of Health.

**Potential conflicts of interest.** D.M.A. reports funding from the UK National Institute for Health Research Grant Number 16/136/111 paid to the institution. M.A.L.M. and S.B.S. report grants or contracts from the World Health Organization made to the institution outside of the submitted work. M.K. received equipment, materials, drugs, medical writing, gifts or other services from the National Institute for Health Research (NIHR) outside of the submitted work. All other authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

**References**

1. O’Neil J. Review on Antibiotic resistance. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance, 2014. Available at: https://asm-review.org/sites/default/files/AMR Review Paper - Tackling a crisis for the health and wealth of nations.pdf. Accessed 10 June 2021.
2. Jassovský D, Littmann J, Zorzet A, Cariño B. Antimicrobial resistance—a threat to the world’s sustainable development. Ups J Med Sci 2016; 121:159-64.
3. Peacock SJ, Parkhill J, Brown NM. Changing the paradigm for hospital outbreak detection by leading with genomic surveillance of nosocomial pathogens. Microbiology (Reading) 2018; 164:1213-9.
4. World Health Organization. Global Antimicrobial Resistance Surveillance System (GLASS): molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System. No. WHO/WSI/AMR/2019.1. Geneva: World Health Organization, 2019.
5. World Health Organization. WHONET software. Geneva: WHO, 2021.
6. SaTSCan. Software for the spatial, temporal, and space-time scan statistics. [https://www.satscan.org/]. Accessed 10 June 2021.
7. WHO Collaborating Centre for Surveillance of Antimicrobial Resistance. WHONET cluster detection with SaTSCan. 2006. [https://www.who.net/Docs/WHONET%208.clusterc20detection%20and%20SaTScan.doc]. Accessed 10 June 2021.
8. National Center for Health Facility Development. National standards in infection control for healthcare facilities. 2009. [https://doh.gov.ph/sites/default/files/publications/NATIONAL_STANDARDS_IN_INFECTION_CONTROL_FOR_HEALTH.pdf]. Accessed 10 June 2021.
9. World Health Organization. Disease outbreaks. [https://www.who.int/teams/environment-climate-change-and-health-emergencies/disease-outbreaks/]. Accessed 10 June 2021.
10. Harris SR, Cartwright EJP, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of methicillin-resistant Staphylococcus aureus: a descriptive study. Lancet Infect Dis 2013; 13:130-6.
11. Long SW, Linson SE, Saavedra MO, et al. Whole-genome sequencing of human clinical Klebsiella pneumoniae isolates reveals misidentification and misunderstandings of Klebsiella pneumoniae, Klebsiella variicola, and Klebsiella quasipneumoniae. MSphere 2017; 2:e00290-17.
12. Sabat AJ, Budimir A, Nasher D, et al; ESMID Study Group of Epidemiological Markers (ESGEM). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill 2013; 18:20380.
13. Foxman B, Zhang L, Koopman JS, Manning SD, Marris CE. Choosing an appropriate bacterial typing technique for epidemiologic studies. Epidemiol Perspect Innov 2005; 2:10.
14. Galarza PG, Alcaba B, Salcedo C, et al. Emergence of high level azithromycin-resistant Neisseria gonorrhoeae strain isolated in Argentina. Sex Transm Dis 2002; 7:269-76.
15. Argimón S, Goater RJE, et al. Microreact: visualizing and sharing genotyping directly from sequencing reads. Microb Genom; 3:e000131. [https://bitbucket.org/genomicepidemiology/pointfinder_db/src/master/]. Accessed 10 June 2021.
16. Wyres KL, Wick RR, Gorrie C, et al. Identification of Klebsiella capsule synthesis loci from whole genome data. Microb Genom 2016; 2. [https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000131]. Accessed 10 June 2021.
17. Underwood A. BactInspector. [https://github.com/antunderwood/bactinspector]. Accessed 10 June 2021.
18. Low AL, Koziol AG, Manninger PA, Blais B, Carrillo CD. ConFindr: rapid detection of interspecies and cross-species contamination in bacterial whole-genome sequence data. PeerJ 2019; 7:e6995.
19. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWAMEM. arXiv 1303.3997. [Preprint]. May 26, 2013 [cited 2021 Jun 10]. Available from: https://arxiv.org/abs/1303.3997.
20. Carattoli A, Zankert E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014; 58:8895-903.
21. Huang SS, Yokoe DS, Stelling J, et al. Automated detection of infectious diseases outbreaks in hospitals: a retrospective cohort study. PLoS Med 2010; 7:e1000238.
22. Fontana I, Bonura E, Lyski Z, Messer W. The brief case: Klebsiella variicola—identifying the misidentified. J Clin Microbiol 2019; 57:e00826-18.
23. Wyres KL, Wick RR, Gorrie C, et al. Identification of Klebsiella capsule synthesis loci from whole genome data. Microb Genom 2016; 2. [https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000131]. Accessed 10 June 2021.
24. Underwood A. epithlib.com/genomicepidemiology/pointfinder db/src/master/. Accessed 10 June 2021.
25. Minh BQ, Schmidt HA, Chenomor O, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 2020; 37:1530-4.
26. Pennington S, Richardson M, Oldfield S. Global Antimicrobial Resistance Surveillance System (GLASS): molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System. No. WHO/WSI/AMR/2019.1. Geneva: World Health Organization, 2019.
27. World Health Organization. WHONET software. Geneva: WHO, 2021.
28. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 2016; 32:3047-8.
29. Babraham Bioinformatics. FastQC. [https://www.bioinformatics.babraham.ac.uk/projects/fastqc/]. Accessed 10 June 2021.
30. Tijet N, Faccone D, Rapoport M, et al. Molecular characteristics of mcr-1-carrying plasmids and new mcr-1 variant recovered from polyclonal clinical Escherichia coli from Argentina and Canada. PLoS One 2013; 8:e63479.
31. Underwood A. BactInspector. [https://github.com/anunderwood/bactinspector]. Accessed 10 June 2021.
32. Low AL, Koziol AG, Manninger PA, Blais B, Carrillo CD. ConFindr: rapid detection of intraspecies and cross-species contamination in bacterial whole-genome sequence data. PeerJ 2019; 7:e6995.
33. Genomic Epidemiology. pointfinder_db. [https://bitbucket.org/genomicepidemiology/pointfinder_db/src/master/]. Accessed 10 June 2021.
34. The Centre of Genomic Pathogen Surveillance. Pathogenwatch | a global platform for genomic surveillance. 2018. [https://pathogenwatch.org/]. Accessed 10 June 2021.
35. Underwood A. BactInspector. [https://github.com/anunderwood/bactinspector]. Accessed 10 June 2021.
36. Hunt M, Mather AE, Sánchez-Busó L, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom 2017; 3:e000131.
37. Wang D, Seo Y, Fan S, et al. Wolf-PLS: a new software for multiple tools and samples in a single report. Bioinformatics 2016; 32:1350-4.
38. G担忧 A, 卓S, 袁M, 营C, 等. 《基因组药敏研究》. 2018. Im Internet: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.
39. Jones RN. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. Clin Infect Dis 2010; 51 Suppl 1: S81-7.
40. Poddusch R, Ullmann M. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998; 11:589-603.
41. Chou A, Roz M, Evangelista MA, et al. Emergence of Klebsiella pneumoniae ST273 carrying bla NDM-7 and ST656 carrying bla NDM-1 in Manila, Philippines. Microb Drug Resist 2016; 22:585-8.
42. Argimón S, Abudahab K, Groter RJ, et al. MiCroreact: visualizing and sharing data for genomic epidemiology and phylogeography. Microb genomics 2016; 2:e000093.
46. Wachino J, Yamane K, Shibayama K, et al. Novel plasmid-mediated 16S rRNA Methylase, RmtC, found in a proteus mirabilis isolate demonstrating extraordinary high-level resistance against various aminoglycosides. Antimicrob Agents Chemother 2006; 50:178 LP-184.

47. David S, Reuter S, Harris SR, et al; EuSCAPE Working Group; ESGEM Study Group. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. Nat Microbiol 2019; 4:1919-29.

48. Ferrari C, Corbella M, Gaiarsa S, et al. Multiple Klebsiella pneumoniae KPC clones contribute to an extended hospital outbreak. Front Microbiol 2019; 10:2767.

49. Jiang HX, Song L, Liu J, et al. Multiple transmissible genes encoding fluoroquinolone and third-generation cephalosporin resistance co-located in non-typhoidal Salmonella isolated from food-producing animals in China. Int J Antimicrob Agents 2014; 43:242-7.

50. Yan JJ, Wang MC, Zheng PX, Tsai JJ, Wu JJ. Associations of the major international high-risk resistant clones and virulent clones with specific ompK36 allele groups in Klebsiella pneumoniae in Taiwan. New Microbes New Infect 2015; 5:1-4.

51. Agyepong N, Govinden U, Owusu-Ofori A, et al. Genomic characterization of multidrug-resistant ESBL-producing Klebsiella pneumoniae isolated from a Ghanaian teaching hospital. Int J Infect Dis 2019; 85:117-23.

52. Ocampo AM, Cienfuegos A, Chen L, et al. High frequency of non-ST258 Klebsiella pneumoniae harboring KPC in tertiary-care hospitals from Medellin-Colombia. https://www.escmid.org/escmid_publications/escmid_elibrary/material/?mid=25419. Accessed 10 June 2021.

53. Aires CAM, Pereira PS, Rocha-de-Souza CM, Silveira MC, Carvalho-Assef APD, Asensi MD. Population structure of KPC-2-producing Klebsiella pneumoniae isolated from surveillance rectal swabs in Brazil. Microb Drug Resist 2020; 26:652-60.

54. Holt KE, Wertheim H, Zadoks RN, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A 2015; 112:E3574-81.

55. Pons MJ, Mari-Almirall M, Ymaña B, et al. Spread of ST348 Klebsiella pneumoniae producing NDM-1 in a Peruvian Hospital. Microorganisms 2020; 8:1392.

56. Natale A, Stelling J, Meledandri M, Messenger LA, D’Ancona F. Use of WHONET-SaTScan system for simulated real-time detection of antimicrobial resistance clusters in a hospital in Italy, 2012 to 2014. Euro Surveill 2017; 22:30484.

57. Latash J, Greene SK, Stavinsky F, et al. Salmonellosis outbreak detected by automated spatiotemporal analysis—New York City, May–June 2019. Morb Mortal Wkly Rep 2020; 69:815.