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Clones and Clusters of Antimicrobial-Resistant *Klebsiella* from Southwestern Nigeria

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40-word summary: We performed whole genome sequencing (WGS) of 141 *Klebsiella* isolated in 2016-2018 at 3 antimicrobial-resistance (AMR) sentinel surveillance tertiary hospitals in southwestern Nigeria. This study uncovered known and novel *K. pneumoniae* lineages circulating in Nigeria that include multidrug-resistant ESBL producers.

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ABSTRACT

Introduction: *Klebsiella pneumoniae* is a World Health Organization high-priority antibiotic-resistant pathogen. However, little is known about the population structure and evolution of *Klebsiella* circulating in Nigeria.

Methods: We performed whole genome sequencing (WGS) of 141 *Klebsiella* isolated between 2016 and 2018 from clinical specimens at 3 antimicrobial-resistance (AMR) sentinel surveillance tertiary hospitals in southwestern Nigeria. We conducted *in silico* multilocus sequence typing, AMR gene, virulence gene, plasmid, and K and O loci profiling, as well as phylogenetic analyses, using publicly available tools and Nextflow pipelines.

Results: Phylogenetic analysis revealed that the majority of the 134 *K. pneumoniae* and 5 *K. quasipneumoniae* isolates from Nigeria characterized are closely related to globally disseminated multidrug-resistant clones. Of the 39 *K. pneumoniae* sequence types (STs) identified, the most common were ST307 (15%), ST5241 (12%), ST15 (~9%), and ST25 (~6%). ST5241, one of 10 novel STs detected, is a single locus variant of ST636 carrying *dfrA14*, *tetD*, *qnrS*, and *oqxAB* resistance genes. The extended-spectrum β lactamase (ESBL) gene $bla_{CTX_M\text{-}15}$ was seen in 72 % of *K. pneumoniae* genomes, while 8% encoded a carbapenemase. Four likely outbreak clusters from one facility, within STs 17, 25, 307, and 5241, were ESBL but not carbapenemase-bearing clones.

Conclusion: This study uncovered known and novel *K. pneumoniae* lineages circulating in Nigeria that include multidrug-resistant ESBL producers. Carbapenemase-producing isolates remain uncommon. WGS retrospectively identified outbreak clusters, pointing to the value of genomic approaches in AMR surveillance for improving infection prevention and control in Nigerian hospitals.

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ABBREVIATIONS

OAU: Obafemi Awolowo University Teaching Hospital

NCDC: Nigeria Centre for Disease Control

LAU: Ladoke Akintola University Teaching Hospital

UCH: University College Hospital

GLASS: Global Antimicrobial Resistance Surveillance System

AMR: Antimicrobial resistance

ENA: European Nucleotide Archive

WGS: Whole Genome Sequencing

CLSI: Clinical & Laboratory Standards Institute

ESBL: Extended Spectrum beta-lactamase

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CONFLICT OF INTEREST

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Main manuscript

INTRODUCTION

Klebsiella is a ubiquitous gram-negative genus that can cause a variety of opportunistic infections [1, 2]. *Klebsiella pneumoniae,* the species most commonly associated with human disease, is frequently implicated in life-threatening bacteremia and sepsis arising from translocations from non-sterile niches or medical devices in hospitals [3]. Although the precise burden of infectious diseases resulting from *Klebsiella* spp. is unknown in Africa, there is an upward trend in reports of *K. pneumoniae*-associated bloodstream infections, including from Nigeria, Côte d'Ivoire, Malawi, Gambia, and South Africa [4-8]. There have also been isolated case reports of non-*pneumoniae* infections in Nigeria [9]. Altogether, these infections have high mortality rates and incur high costs [10, 11].

Rigorous surveillance of *Klebsiella* clones circulating within Nigerian hospitals and communities is needed to inform treatment guidelines and prioritize AMR interventions. Whole genome sequencing (WGS) offers the attractive prospect of meeting this need within local resource constraints. WGS tools also provide taxonomic resolution below serotype levels, a feat impossible in resource-limited settings by classical typing methods [13]. Mapping the diversity of *Klebsiella* populations at high resolution, with spatiotemporal dynamics, also makes it possible to elucidate the origin and dissemination of AMR [12, 13].

In contrast to similarly constrained settings elsewhere in Africa [14–16], few Nigeria reports include in-depth analysis of more than a handful of *Klebsiella* isolates. For example, as of the time of writing, Pathogenwatch, which sources its public genomes from ENA, includes only 109 *Klebsiella* genomes from Nigeria [17]. Nigeria recently initiated national AMR surveillance following commissioning of a 2017 National Action Plan, and the Nigeria Centre for Disease Control (NCDC) enrolled the country in the WHO's Global Antimicrobial Resistance Surveillance System (GLASS) [18]. NCDC is building a network that currently consists of 9 AMR sentinel hospital laboratories and 2 national reference laboratories [19]. The very low number of *Klebsiella* genomes from Nigeria compelled the

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Nigeria node of the Global Health Research Unit (GHRU) on Genomic Surveillance of AMR, which provides WGS services to the reference laboratories, to collect and sequence retrospective isolates. In addition to increasing the number of relevant genomes in publicly available databases that can support prospective surveillance, this study aimed to understand the population structure, evolution, pathogenicity, and transmission dynamics of this critical-priority pathogen circulating in southwestern Nigeria.

METHODS

Ethical Considerations

Most genomes included in this study were acquired from surveillance without linked patient data. We additionally integrated data from the Obafemi Awolowo University (OAU) Teaching Hospital sentinel site, as approved by the Ethics and Research Committee, Obafemi Awolowo University Teaching Hospitals Complex with registration number ERC/2017/05/06.

GHRU Nigeria Workflow

Strains, re-identification, and antimicrobial susceptibility testing: The NCDC, Nigeria's AMR surveillance coordinating center, requested from the network cryopreserved invasive isolates (from blood, cerebrospinal fluid, and urine) from 2016-2018, to develop a local genome database. Sentinel laboratories provided the national reference laboratory with strains and information on species identity, clinical diagnosis, and antimicrobial susceptibility tests (AST) via WHONET [20]. At the national reference laboratory, in collaboration with the GHRU, isolate identification and AST were validated using the VITEK system (version 2.0), testing, as appropriate, amikacin, gentamicin, ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, cefuroxime_axetil, cefepime, ceftriaxone, cefoperazone/sulbactam, nitrofurantoin, nalidixic acid, ciprofloxacin, ertapenem, imipenem, meropenem, trimethoprim-sulphamethoxazole, colistin, and tigecycline [21]. Antimicrobial susceptibility test results were interpreted in line with the CLSI standards [22].

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DNA extraction, Library Preparation, and Sequencing: Genomic DNA was extracted using the Wizard DNA extraction kit (Promega; Wisconsin, USA; Cat. No: A1125). DNA was quantified using a dsDNA Broad Range fluorometric quantification assay (Invitrogen; California, USA; Cat. No: Q32853). Double-stranded DNA libraries (avg. 500 bp) were prepared using the Covaris LC220 for fragmentation, and NEBNext Ultra II FS DNA library kit for Illumina with 384-unique indexes (New England Biolabs, Massachusetts, USA; Cat. No: E6617L). Libraries were sequenced using the HiSeq X10 with 150 bp paired-end chemistry (Illumina, CA, USA).

Genome assembly: Genome assembly was carried out according to the GHRU protocol (https://www.protocols.io/view/ghru-genomic-surveillance-of-antimicrobial-resista-bpn6mmhe). Parameters for post-assembly quality checks include the total genome size (between 4584497 bp to 7012008 bp), N50 score (> 25000), contaminant level (< 5%), and number of contigs (< 300). Only *Klebsiella* genomes that passed all quality checks (*K. pneumoniae*, n = 134; *K. quasipneumoniae*, n = 5) were selected for downstream analysis.

Single Nucleotide Polymorphism (SNP) Analysis

Sequence reads were mapped to the reference genome of the *K. pneumoniae* strain NTUH-K2044 (Genbank Accession number GCF_009497695.1) to determine evolutionary relationships among the isolates according to GHRU protocol (https://www.protocols.io/view/ghru-genomic-surveillance-of-antimicrobial-resista-bpn6mmhe). Pairwise SNP distances for likely outbreak isolates from OAU were calculated from the pseudo-genome alignment using FastaDist (https://gitlab.com/antunderwood/fastadist). Outbreak isolates belonging to STs 17, 25, and 307 were aligned to reference genomes NZ_CP009461.1, NZ_CP031810.1, and NZ_CP022924.1 respectively, selected with Bactinspector (https://gitlab.com/antunderwood/bactinspector/). The ST636 reference EuSCAPE_UK136 was selected from Pathogenwatch collections, being the only known single locus variant of ST5241.

Prediction of AMR Determinants, Virulence Factors, and Plasmid Profiling

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AMR determinants and plasmid replicons were predicted in accordance with the aforementioned GHRU protocol. *omp* mutations, virulence score, and acquired virulence genes were determined using Kleborate (v2.0.0; https://github.com/katholt/Kleborate) [23, 24].

Concordance Analysis

Concordance between phenotypic and genotypic AMR for aminoglycosides, β-lactams, carbapenem, cephalosporin, quinolone, and trimethoprim was determined by using https://gitlab.com/-/snippets/2050300 based on a protocol (https://glcdn.githack.com/cgps/ghru/ghrur/raw/master/docs/articles/simple_concordance_example.html) that employs the epi.tests function of the epiR package to calculate sensitivity and specificity with confidence bounds.

Identification of Multilocus Sequence Types.

Multilocus sequence types (MLSTs) according to the Pasteur scheme were determined by following the aforementioned GHRU protocol (https://www.protocols.io/view/ghru-genomic-surveillance-of-antimicrobial-resista-bpn6mmhe) [25]. We also used Kleborate (v2.0.0; https://github.com/katholt/Kleborate) to identify the ST most closely related to novel STs (in terms of locus variants).

Availability of Sequence Data

Raw sequence datasets generated during this study have been deposited in the ENA with bioproject number PRJEB29739 (https://www.ebi.ac.uk/ena/browser/view/PRJEB29739). Accessions are available in Supplementary Table 3.

RESULTS

Epidemiology, Sequence Types, Virulence-Associated Determinants, and AMR Determinants of *Klebsiella* from Southwestern Nigeria Hospitals

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Retrospective *Klebsiella* isolates were collected from 3 hospitals that had archival isolates: Obafemi Awolowo University Teaching Hospital (OAU, Ile-Ife, Osun State, Nigeria; n = 92), University College Hospital (UCH, Ibadan, Oyo State, Nigeria; n = 30), and Ladoke Akintola University Teaching Hospital (LAU, Oshogbo, Osun State, Nigeria; n = 12). Of the 134 isolates, 87 were from blood, comprising 45 from OAU, 30 from UCH, and 12 from LAU. We additionally sequenced 47 non-blood isolates from OAU, recovered from urine (n = 37), ocular (n = 2), stool (1), throat (n = 2), and rectal (n = 5) swabs.

Of the 134 genomes confirmed as *K. pneumoniae* by WGS, 18 (13%) and 33 (25 %) were identified by reference laboratory VITEK and the sentinel laboratories, respectively, as either other members of the *Enterobacteriaceae* family, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa* (Supplementary Table 1). All WGS-identified *Klebsiella quasipneumoniae* isolates were identified either as *K. pneumoniae* or *Enterobacter* complexes by VITEK and the sentinel laboratories, respectively (Supplementary Table 1). The phylogenetic tree, epidemiological data, and analyses data can be visualized in Microreact for retrospective *Klebsiella pneumoniae* (https://microreact.org/project/GHRUNigeriaKpneumoniae/2a856694), and OAU outbreak isolates (ST17: https://microreact.org/project/brfq17BXzwmqptfcrNRfZR/71b2f0f1; ST25: https://microreact.org/project/8FP4F1D5fSQMv6FDxbR39b/bd381f0b; ST307: https://microreact.org/project/sV5NsJ8szcorvFAeFQgV4E/6498edf1; ST5241: https://microreact.org/project/u5RPX7CjyitjRYMQ9ByW4W/1a3570e2).

Overall, the *K. pneumoniae* isolates sequenced in this study belonged to 39 different sequence types (STs), with ST307, ST5241, ST15, and ST25 being most common (Figure 1). Only 4 STs (ST15, ST101, ST147, and ST307) were found across the 3 sentinel sites (Figure 1). *Klebsiella quasipneumoniae* genomes (n = 5) belonged to 4 STs: ST1602, ST2133, ST5250 (n = 1 each), and ST5249 (n = 2).

We identified few virulence genes associated with invasiveness, including yersiniabactin (*ybt* genes and *fyuA*; n = 42, 31.34%), aerobactin (*iuc*; n = 2), salmochelin (*iro*; n = 2), and capsule expression upregulators *rmpADC* and *rmpA2* (n = 2). Only 2 ST86 isolates had a virulence score of 3, the highest

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recorded in our collection. No acquired *K pneumoniae* virulence gene was detected in novel ST5241 (Figure 2), nor in the non-pneumoniae *Klebsiella* species.

We detected 33 different phenotypically-defined K (capsular) loci from the invasive *K. pneumoniae* isolates sequenced, with KL102, KL123, KL2, KL62, and KL27 representing the 5 most common K loci (Supplementary Table 2). Eleven different O loci were detected among the invasive *K. pneumoniae* isolates recovered from bloodstream infections. Collectively, the 3 O loci – O1v1, O2v2, and O1v2 – accounted for more than 65% of the *K. pneumoniae* strains, and O5 was solely detected in ST5241 genomes.

Genes conferring resistance or reduced susceptibility to at least 5 antibiotic classes were detected in 116 (86.6%) of *K. pneumoniae* genomes. Aside from core bla_{SHV}, these included8 β-lactamase genes, of which the extended-spectrum β-lactamase (ESBL) $bla_{CTX\text{-}M\text{-}15}$ was by far the most common, present in 71.6% (n = 96) of the *K. pneumoniae* isolates belonging to 33 STs (Figure 2). As seen in other studies, we found this ESBL in strains with plasmids belonging to IncFIB_K (n = 70/96), IncFII_K (n = 51/96), and IncR (n = 43/96) incompatibility groups [26, 27]. The $bla_{NDM\text{-}1}$ (n = 8/134), $bla_{NDM\text{-}5}$ (n = 2/134), and $bla_{OXA\text{-}48}$ (n = 1/134) genes were the only carbapenemases detected, and these occurred in 8 STs, including ST392 ($bla_{NDM\text{-}1}$; n = 2), ST147/147-1-locus-variant ($bla_{NDM\text{-}1}$; n = 2), ST530 ($bla_{NDM\text{-}5}$; n = 2), ST43, ST15, ST307, ST716 ($bla_{NDM\text{-}1}$; n = 1 each), and ST219 ($bla_{OXA\text{-}48}$; n = 1) (Figure 2). OmpK35 truncations were observed together with $bla_{CTX\text{-}M\text{-}15}$ in 11 isolates and with $bla_{NDM\text{-}1}$ in 5 isolates. Altogether, 18 (13.5%) *K. pneumoniae* strains carried carbapenemase genes, and/or ESBL genes plus porin defects known to confer carbapenem resistance [28]. Phenotypic antimicrobial susceptibility testing found 11 of 128 isolates carbapenem non-susceptible, and $bla_{NDM\text{-}1}$ (n = 6), $bla_{NDM\text{-}5}$ (n = 2), and/or $bla_{CTX\text{-}M\text{-}15}$ and OmpK35 truncations (n = 1) could account for non-susceptibility in 9 of them (Figure 2). Concordance analysis showed that phenotypic AST and genotypic AMR gene prediction results agree substantially for carbapenems (concordance: 92.2%; sensitivity: 82% [48.22% - 97.72%]; specificity: 93.2% [86.97% - 97%]).

Reduced susceptibility to nalidixic acid (n = 70/125) or ciprofloxacin (n = 114/125) could be explained by the presence of one or more combinations of mutations in the quinolone resistance

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determining region (QRDR) of *gyrA* and *parC* (n = 60), plasmid-mediated quinolone resistance genes (*qnrS*, *qnrA*, *qnrB*; n = 82), and/or efflux-mediating mutations in *acrR* gene (n = 98) (Figure 2). The 5 *K. quasipneumoniae* were resistant to phenicols, tetracyclines, sulphonamides, trimethoprim, and β-lactams (https://microreact.org/project/4f6Y56ECE979wkj8oDBhk2/dee16dac). One LAU isolate, collected 6 months after an outbreak of *K. quasipneumoniae* strains carrying bla_{NDM-5} in Abuja, Nigeria, was remotely related to those outbreak strains (SNP distance ≥ 235), but, like the other 4 *K. quasipneumoniae* from the current study, it lacked carbapenemase genes [9].

Retrospective Characterization of Possible Outbreaks

Recovery dates for 74 of 92 isolates from the OAU facility were supplied. In under a year, between 6 and 14 isolates were recovered belonging to ST17, ST25, ST307, and ST5241. We noticed clusters of closely related isolates that were recovered within short periods, and we hypothesized that these might represent unreported/undetected outbreaks. Intra-cluster pairwise distances within the potential outbreak clades ranged from 1 to 3 SNPs, and inter-cluster pairwise distances within the same ST ranged from 46 to 370. Temporally-clustered, closely-related clonal groups within each of the four STs (Figure 3), with similar AMR gene, virulence gene, and plasmid replicon profiles, support that these clusters represent outbreaks, and that nosocomial transmission contributes heavily to invasive *Klebsiella* infections. The outbreaks mostly involved ESBL lineages but no carbapenemase-producers.

DISCUSSION

This report on the population structure of *Klebsiella* in 3 tertiary hospitals addresses critical knowledge gaps regarding the characteristics of *K. pneumoniae* in Nigeria and presents 5 *K. quasipneumoniae* genomes, which without WGS cannot be differentiated from *K. pneumoniae* in our setting, even at reference laboratory level. Ours and other data reveal that multiple *K. quasipneumoniae* lineages, including resistant ones, circulate in Nigeria [9]. We also corroborate

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previous reports on the diversity of *K. pneumoniae*, which pose challenges as well as opportunities for curtailing and combating this pathogen [29, 30].

The high prevalence genes conferring resistance to fluoroquinolones and extended-spectrum β-lactams, last-line options for Nigeria's least affluent patients, in the most common lineages, reported here is of concern in spite of relatively low rates of carbapenem resistance in our setting, or elsewhere in Africa, compared to other countries [15, 31, 32, 33]. Moreover, while uncommon in this study, ST147 and ST392 strains bearing bla_{NDM-1}, and ST101 strains carrying bla_{OXA-48}were documented [37]. All these lineages deserve close and careful monitoring in national prospective surveillance, and further work is necessary to identify and track mobile elements they carry. The preponderance of plasmids in the *Klebsiella* strains sequenced reflects the known propensity of this genus to harbor and diversify mobile elements in the face of high selective pressure and has implications for resistance in the many species to which *K. pneumoniae* transmits DNA [2, 34].

Clonal group (CG) 307 is believed to be displacing the notorious CG258 as a primary lineage in South Africa, Italy, Colombia, and the USA, and it may be more virulent [31, 36-40]. The high ST307 frequency here, and in Malawiand South Africa, may explain the low overall prevalence of carbapenem resistance we observed compared to other locations [14, 17, 32, 35]. We recorded 21 ST307 isolates, but no ST258 isolates, and only 7 CG258 isolates —5 ST340 and 2 ST11.

Clusters of likely clonal isolates within clonal complexes 15, 25, 17, ST5241, 392, and 101 were identified among retrospective isolates from OAU, the most sampled sentinel site, within clonal complexes, 15, 25, and 17, as well as ST307, ST5241, 392, and 101. The close genetic distances observed within 4 of these clones (< 4 pairwise SNP differences in each case) and the similar or identical resistance, virulence, and plasmid replicon profiles, and temporal clustering of their isolation strongly suggest that each of these clusters represents a retrospectively identified outbreak. As fewer retrospective isolates were stored at the other two institutions, we cannot rule out similar occurrences that are below our limits of detection. Outbreaks were caused by highly resistant lineages such as the ST17 cluster, but also more sensitive ones like the ST5241 and ST307 *K. pneumoniae* clusters. While outbreaks can sometimes be detected by phenotypic testing in diagnostic labs alone, the minimal biochemical and narrow disc diffusion test repertoires employed at sentinel labs, which underlie many

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species' misidentifications (Supplementary Table 1), make detection unlikely when the number of outbreak isolates is small. Our data show that, as in countries with more intensive surveillance, outbreaks likely commonly occur within health facilities in Nigeria and elsewhere in West Africa and will typically be missed without genomic support [7, 12, 42, 43]. OAU and UCH currently provide subsidized blood culture for many patients, but most Nigerian facilities with blood culture do not, and health insurance coverage is low. Therefore, identifying outbreaks is likely to be curtailed by patients' inability to pay. Our data reveal that recent efforts to strengthen Nigeria's AMR response and boost infection prevention and control could be mutually enhancing if blood culture of routine febrile patient blood culture is facility-supported, and if prospective genomic surveillance is used.

This study has some limitations. First, very few archival isolates from only 3 facilities were available, and epidemiological data were incomplete. Furthermore, the sequence types observed here were sampled only from invasive infections. Nonetheless, this study represents an important starting point for documenting *Klebsiella* lineages, and we will build on these findings with prospective surveillance at more hospitals in southwestern Nigeria, which have now been enrolled in the national surveillance system.

CONCLUSION

We have detailed the characteristics of *Klebsiella* isolates from 3 southwestern hospitals in Nigeria. In addition to incorporating Nigeria-derived data into global phylogeographies of multidrug-resistant *K. pneumoniae*, our findings point to the significance of ST307 in Nigeria. Carbapenem-resistant STs 147 and 392, as well as novel ST5241 *K. pneumoniae*, may also represent future surveillance priorities for southwestern Nigeria. We have shown the benefits of public health genomic surveillance of pathogens — in particular, the potential for outbreak identification. Infection prevention and control is a key pillar of Nigeria's AMR National Action Plan and the data from this paper suggest that genomic surveillance and IPC implementation could be mutually reinforcing in this collaboration among scientists, health institutions, and the public health authority in Nigeria.

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REFERENCES

1. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev*. **1998**; 11: 589–603.

2. Wyres KL, Holt KE. *Klebsiella pneumoniae* Population Genomics and Antimicrobial-Resistant Clones. *Trends Microbiol*. **2016**; 24: 944–956.

3. Wyres KL, Nguyen TNT, Lam MMC, et al. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. *Genome Med*. **2020**; 12(1): 11.

4. Olalekan A, Onwugamba F, Iwalokun B, Mellmann A, Becker K, Schaumburg F. High proportion of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* among extended-spectrum β-lactamase-producers in Nigerian hospitals. *J Glob Antimicrob Resist* **2020**; 21: 8–12.

5. Müller-Schulte E, Tuo MN, Akoua-Koffi C, Schaumburg F, Becker SL. High prevalence of ESBL-producing *Klebsiella pneumoniae* in clinical samples from central Côte d'Ivoire. *Int J Infect Dis* **2020**; 91: 207–209.

6. Musicha P, Cornick JE, Bar-Zeev N, et al. Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. *Lancet Infect Dis* **2017**; 17: 1042–1052.

7. Okomo U, Senghore M, Darboe S, et al. Investigation of sequential outbreaks of *Burkholderia cepacia* and multidrug-resistant extended spectrum β-lactamase producing *Klebsiella* species in a West African tertiary hospital neonatal unit: a retrospective genomic analysis. *The Lancet Microbe* **2020**; 1: e119–e129.

8. Mbelle NM, Feldman C, Sekyere JO, Maningi NE, Modipane L, Essack SY. Pathogenomics and Evolutionary Epidemiology of Multi-Drug Resistant Clinical Klebsiella pneumoniae Isolated from Pretoria, South Africa. Sci Rep **2020**; 10.

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9. Brinkac LM, White R, D'Souza R, Nguyen K, Obaro SK, Fouts DE. Emergence of New Delhi Metallo-β-Lactamase (NDM-5) in *Klebsiella quasipneumoniae* from Neonates in a Nigerian Hospital. *mSphere* **2019**; 4.

10. Nejad SB, Allegranzi B, Syed SB, Ellisc B, Pittetd D. Health-care-associated infection in Africa: a systematic review. *Bull. World Health Organ*. **2011**; 89: 757–765.

11. Irek EO, Amupitan AA, Obadare TO, Aboderin AO. A systematic review of healthcare-associated infections in Africa: An antimicrobial resistance perspective. *Afr. J. Lab. Med*. **2018**; 7.

12. David S, Reuter S, Harris SR, et al. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. *Nat Microbiol*. **2019**; 4(11): 1919-1929.

13. Perdigão J, Modesto A, Pereira AL, et al. Whole-genome sequencing resolves a polyclonal outbreak by extended-spectrum beta-lactam and carbapenem-resistant *Klebsiella pneumoniae* in a Portuguese tertiary-care hospital. *Microb Genomics* **2020**; mgen000349.

14. Musicha P, Msefula CL, Mather AE, et al. Genomic analysis of *Klebsiella pneumoniae* isolates from Malawi reveals acquisition of multiple ESBL determinants across diverse lineages. *J Antimicrob Chemother* **2019**; 74: 1223–1232.

15. Sonda T, Kumburu H, van Zwetselaar M, et al. Molecular epidemiology of virulence and antimicrobial resistance determinants in *Klebsiella pneumoniae* from hospitalised patients in Kilimanjaro, Tanzania. *Eur J Clin Microbiol Infect Dis* **2018**; 37: 1901–1914.

16. Rakotondrasoa A, Passet V, Herindrainy P, et al. Characterization of *Klebsiella pneumoniae* isolates from a mother-child cohort in Madagascar. *J Antimicrob Chemother* **2020**; 75: 1736–1746.

17. Argimón S, David S, Underwood A, et al. Rapid Genomic Characterization and Global Surveillance of *Klebsiella* Using Pathogenwatch. Clin Infect Dis 2021;73: In this supplement

18. Nigeria Centre for Disease Control (NCDC). National Action Plan for Antimicrobial Resistance 2017-2022. 2017. Available at: https://ncdc.gov.ng/themes/common/docs/protocols/77_1511368219.pdf. Accessed June 10, 2021.

19. National Centre for Disease Control. NCDC Annual Report 2018. 2019; 1–74. Available at: https://ncdc.gov.ng/themes/common/files/annualreports/18803aba62a09ada4ad84c8db76c22ea.pdf. Accessed June 10, 2021.

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20. O'Brien TF, Stelling JM. WHONET: an information system for monitoring antimicrobial resistance. *Emerg Infect Dis* **1995**; 1: 66.

21. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* **2012**; 18: 268–281.

22. CLSI. M100 Performance Standards for Antimicrobial Susceptibility Testing A CLSI supplement for global application. Performance Standards for Antimicrobial Susceptibility Testing Performance Standards for Antimicrobial Susceptibility Testing. **2020**; Available at: https://www.academia.edu/41587236/M100_Performance_Standards_for_Antimicrobial_Susceptibility_Testing_A_CLSI_supplement_for_global_application_28th_Edition. Accessed June 10, 2021.

23. Lam MMC, Wyres KL, Judd LM, et al. Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. *Genome Med* **2018**; 10: 77.

24. Lam MMC, Wick RR, Wyres KL, et al. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in *Klebsiella pneumoniae* populations. *Microb Genomics* **2018**; 4: e000196.

25. Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* **2005**; 43: 4178–4182.

26. Irrgang A, Falgenhauer L, Fischer J, et al. CTX-M-15-producing *E. coli* isolates from food products in Germany are mainly associated with an IncF-type plasmid and belong to two predominant clonal *E. coli* lineages. *Front Microbiol* **2017**; 8.

27. Agyekum A, Fajardo-Lubián A, Ansong D, Partridge SR, Agbenyega T, Iredell JR. blaCTX-M-15 carried by IncF-type plasmids is the dominant ESBL gene in *Escherichia coli* and *Klebsiella pneumoniae* at a hospital in Ghana. *Diagn Microbiol Infect Dis* **2016**; 84: 328–333.

28. Logan LK, Weinstein RA. The epidemiology of Carbapenem-resistant enterobacteriaceae: The impact and evolution of a global menace. *J Infect Dis* **2017**; 215: S28–S36.

29. Brisse S, Fevre C, Passet V, et al. Virulent clones of *Klebsiella pneumoniae*: Identification and evolutionary scenario based on genomic and phenotypic characterization. *PLoS One* **2009**; 4.

bioRxiv preprint doi: https://doi.org/10.1101/2021.06.21.449255; this version posted June 22, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

30. Bialek-Davenet S, Criscuolo A, Ailloud F, et al. Genomic Definition of Hypervirulent and Multidrug-Resistant *Klebsiella pneumoniae* Clonal Groups. *Emerg Infect Dis* **2014**; 20: 1812–1820.

31. Choi M, Hegerle N, Nkeze J, et al. The Diversity of Lipopolysaccharide (O) and Capsular Polysaccharide (K) Antigens of Invasive *Klebsiella pneumoniae* in a Multi-Country Collection. *Front Microbiol* **2020**; 11.

32. Saavedra SY, Bernal JF, Montilla-Escudero E, et al. Complexity of Genomic Epidemiology of Carbapenem-Resistant Klebsiella pneumoniae Isolates in Colombia Urges the Reinforcement of Whole Genome Sequencing-Based Surveillance Programs. Clin Infect Dis 2021;73: In this supplement

33. Nagaraj G, Shammana V, Govindan V, et al. High-Resolution Genomic Profiling of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates: A Multicentric Retrospective Indian Study. Clin Infect Dis 2021;73: In this supplement

34. Wein T, Wang Y, Hülter NF, Hammerschmidt K, Dagan T. Antibiotics Interfere with the Evolution of Plasmid Stability. *Curr Biol* **2020**; 30.

35. Lowe M, Kock MM, Coetzee J, et al. *Klebsiella pneumoniae* ST307 with $bla_{OXA-181}$, South Africa, 2014–2016. *Emerg Infect Dis* **2019**; 25: 739–747.

36. Villa L, Feudi C, Fortini D, et al. Diversity, virulence, and antimicrobial resistance of the KPC-producing *KlebsiellaK pneumoniae* ST307 clone. *Microb Genomics* **2017**; 3: e000110.

37. Geraci DM, Bonura C, Giuffrè M, et al. Is the monoclonal spread of the ST258, KPC-3-producing clone being replaced in southern Italy by the dissemination of multiple clones of carbapenem-non-susceptible, KPC-3-producing *Klebsiella pneumoniae*? *Clin. Microbiol. Infect*. 2015; 21: e15–e17.

38. Ocampo AM, Chen L, Cienfuegos A V, et al. A two-year surveillance in five Colombian tertiary care hospitals reveals high frequency of non-CG258 clones of carbapenem-resistant *Klebsiella pneumoniae* with distinct clinical characteristics. *Antimicrob Agents Chemother* **2016**; 60: 332–342.

39. Castanheira M, Farrell SE, Wanger A, Rolston K V., Jones RN, Mendes RE. Rapid Expansion of KPC-2-Producing *Klebsiella pneumoniae* Isolates in Two Texas Hospitals due to Clonal Spread of ST258 and ST307 Lineages. *Microb Drug Resist* **2013**; 19: 295–297.

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40. Long SW, Olsen RJ, Eagar TN, et al. Population genomic analysis of 1,777 extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates, Houston, Texas: Unexpected abundance of clonal group 307. *MBio* **2017**; 8.

41. Chen L, Mathema B, Pitout JDD, DeLeo FR, Kreiswirth BN. Epidemic *Klebsiella pneumoniae* ST258 is a hybrid strain. *MBio* **2014**; 5.

42. Argimón S, Masim MAL, Gayeta JM, et al. Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines. *Nat Commun* **2020**; 11: 1–15.

43. Carlos CC, Masim MAL, Lagrada ML, et al. Genome Sequencing Identifies Previously Unrecognized *Klebsiella pneumoniae* Outbreaks in Neonatal Intensive Care Units in the Philippines. Clin Infect Dis 2021;73: In this supplement

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FIGURE LEGENDS

Figure 1. Sequence Types of *Klebsiella pneumoniae* and their distribution across the 3 sentinel sites.

Figure 2. Epidemiological data, virulence determinants, antibiotic profile (phenotypic resistance), and antimicrobial resistance determinants in *K. pneumoniae* genomes ordered by phylogeny. The heat map shows the presence (red) or absence (blank) of virulence determinants, phenotypic resistance, AMR genes, and plasmid replicon genes. Tree nodes represent the origin of isolate collection. The shape of the tree nodes depicts the outbreak (star) and non-outbreak (circle) isolates. The data are available at https://microreact.org/project/GHRUNigeriaKpneumoniae/2a856694.

Figure 3: The timeline of likely outbreak of *K. pneumoniae* clones belonging to STs 17 (a), 25 (b), 307(c), and 5241(d) from the OAU sentinel site. The shape of the tree nodes depicts the outbreak (star) and non-outbreak (circle) isolate, while the color of the tree nodes depicts the sentinel site. The data are available at https://microreact.org/project/brfq17BXzwmqptfcrNRfZR/71b2f0f1 (ST17), https://microreact.org/project/8FP4F1D5fSQMv6FDxbR39b/bd381f0b (ST25), https://microreact.org/project/sV5NsJ8szcorvFAeFQgV4E/6498edf1 (ST307), and https://microreact.org/project/u5RPX7CjyitjRYMQ9ByW4W/1a3570e2 (ST5241).

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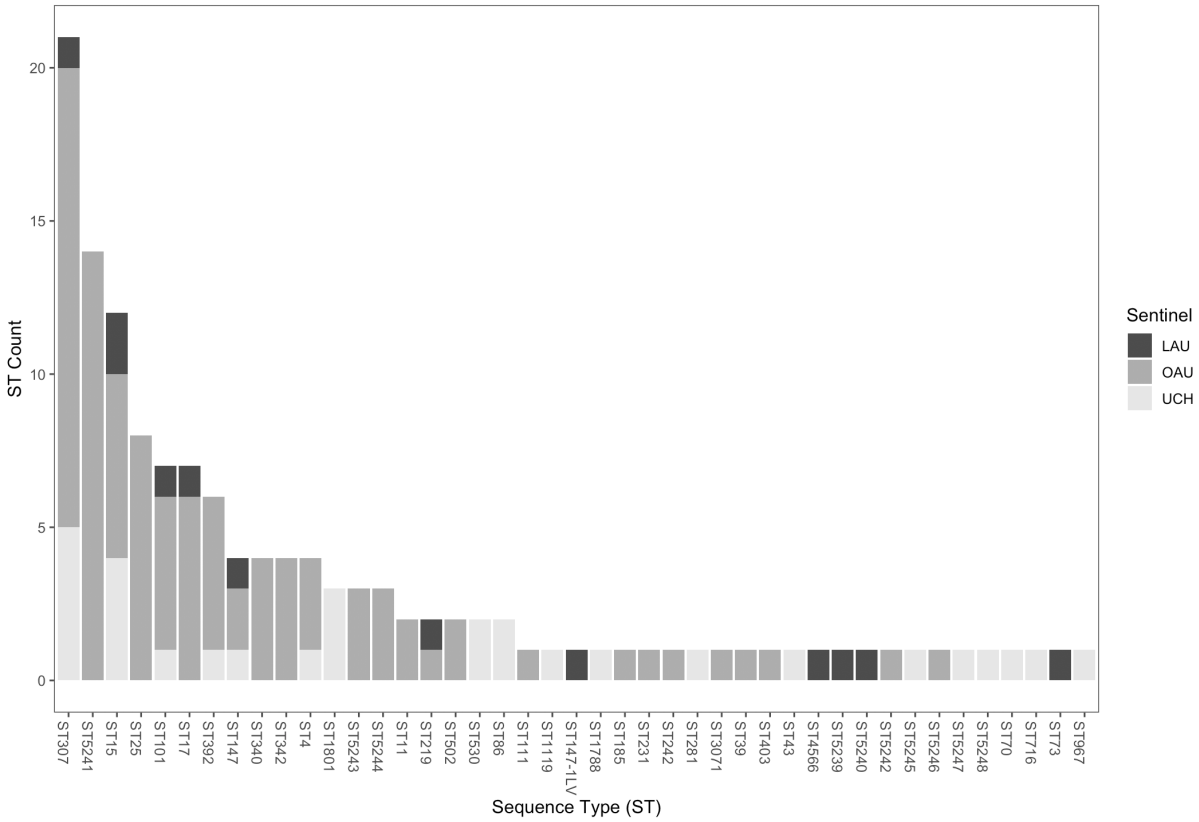
FIGURES

Figure 1.



Key (LAU = Ladoke Akintola University Teaching Hospital, OAU = Obafemi Awolowo University Teaching Hospital, UCH = University College Hospital).

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Figure 2.

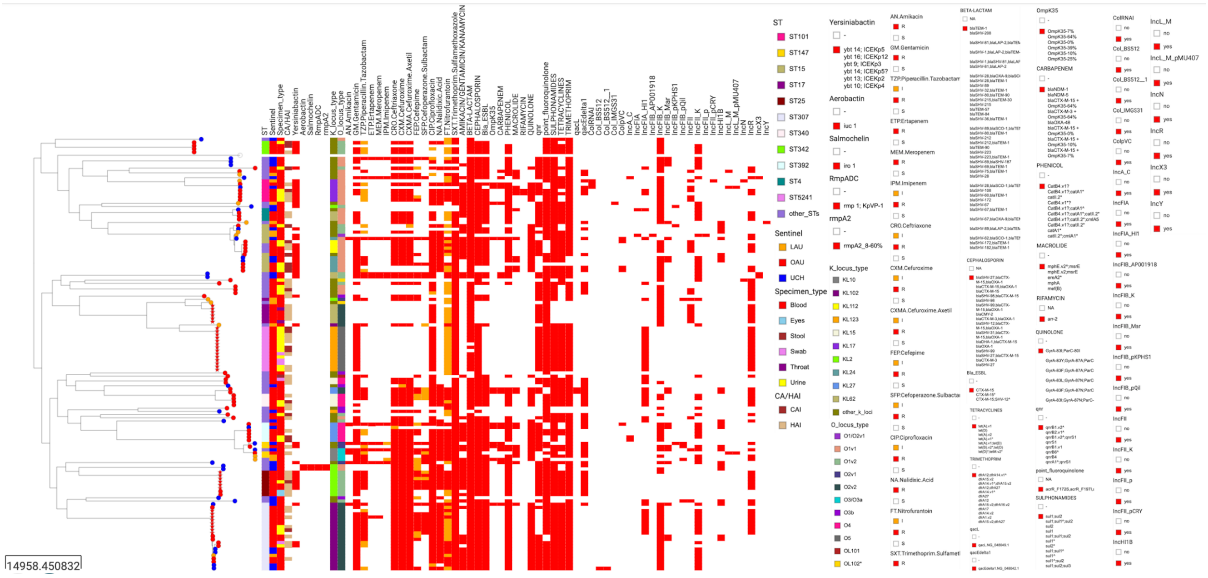

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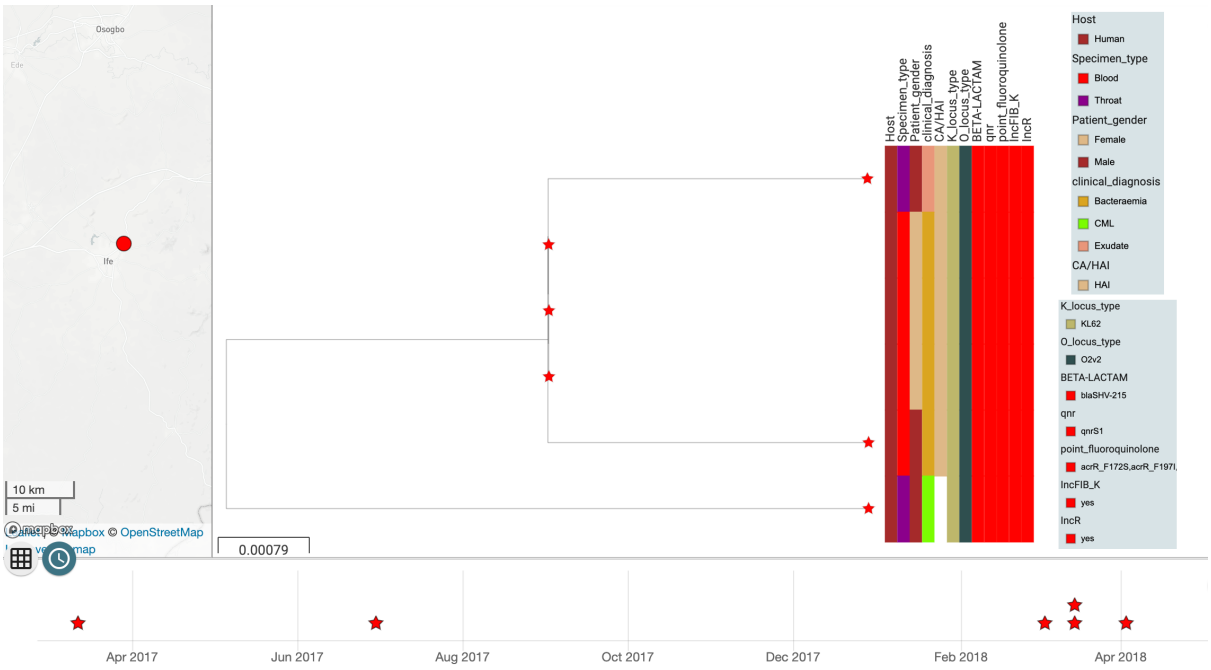
Figure 3:

(a)



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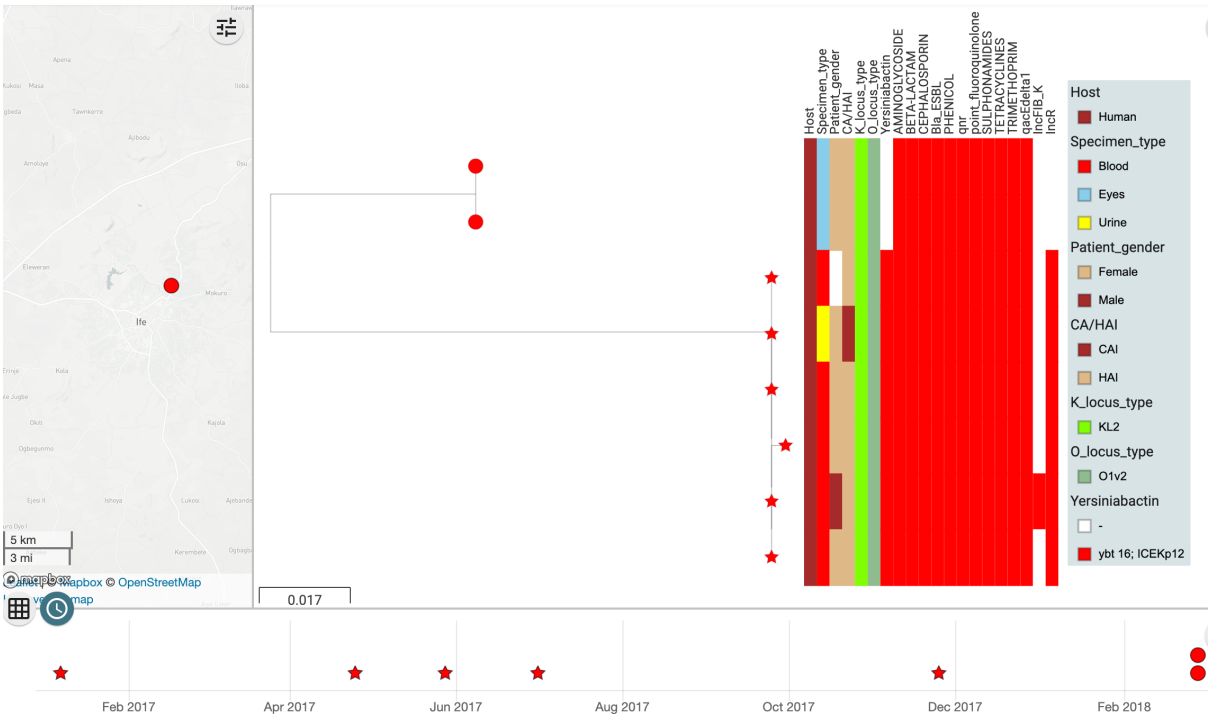

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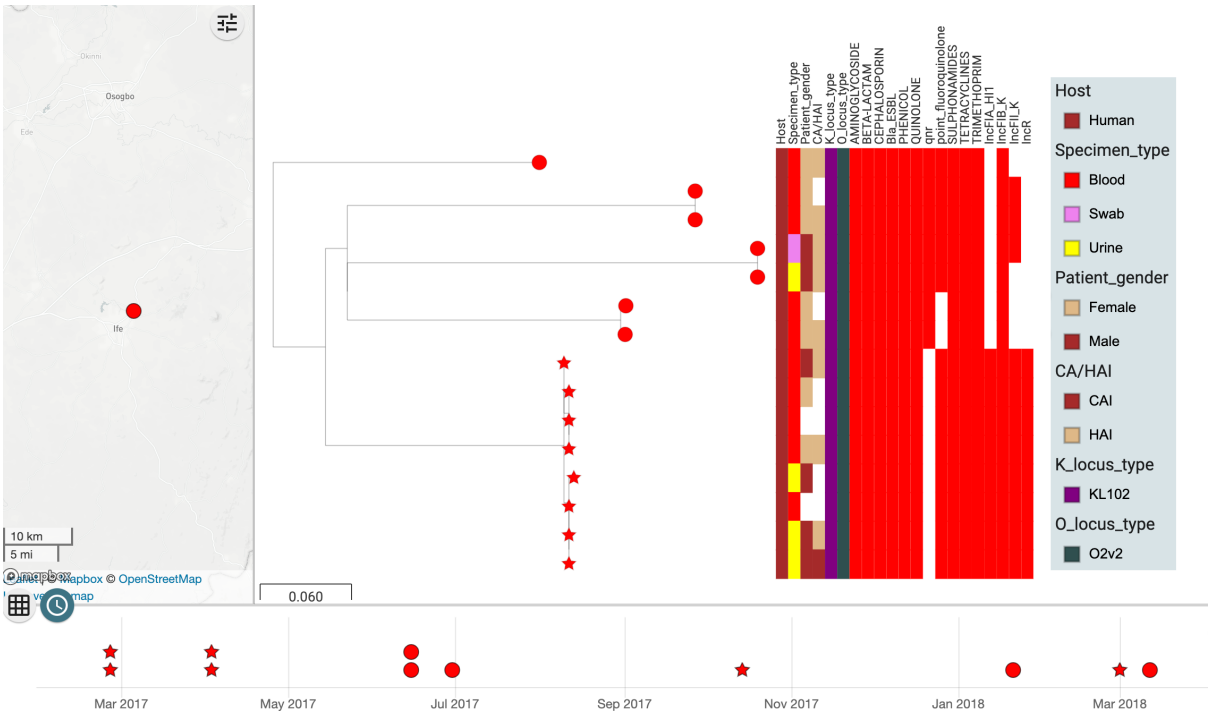
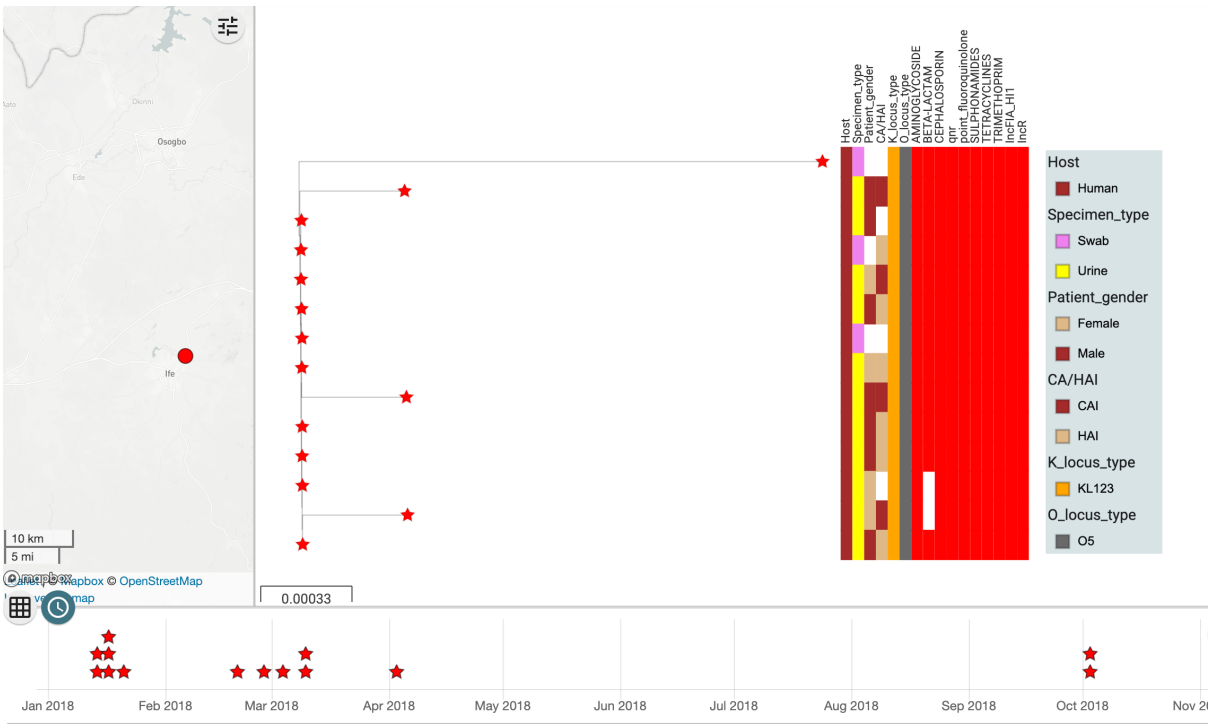
(c)



(d)