

RESEARCH

Open Access



Longitudinal analysis within one hospital in sub-Saharan Africa over 20 years reveals repeated replacements of dominant clones of *Klebsiella pneumoniae* and stresses the importance to include temporal patterns for vaccine design considerations

Eva Heinz^{1,2*} , Oliver Pearse^{2,3}, Allan Zuza³, Sithembile Bilima³, Chisomo Msefula⁴, Patrick Musicha^{2,3}, Patriciah Siyabu⁵, Edith Tewesa⁵, Fabrice E. Graf², Rebecca Lester^{3,6}, Samantha Lissauer^{3,7}, Jennifer Cornick^{3,7}, Joseph M. Lewis^{2,3,7}, Kondwani Kawaza^{3,4}, Nicholas R. Thomson^{8,9} and Nicholas A. Feasey^{2,3,10*}

Abstract

Background Infections caused by multidrug-resistant gram-negative bacteria present a severe threat to global public health. The WHO defines drug-resistant *Klebsiella pneumoniae* as a priority pathogen for which alternative treatments are needed given the limited treatment options and the rapid acquisition of novel resistance mechanisms by this species. Longitudinal descriptions of genomic epidemiology of *Klebsiella pneumoniae* can inform management strategies but data from sub-Saharan Africa are lacking.

Methods We present a longitudinal analysis of all invasive *K. pneumoniae* isolates from a single hospital in Blantyre, Malawi, southern Africa, from 1998 to 2020, combining clinical data with genome sequence analysis of the isolates.

Results We show that after a dramatic increase in the number of infections from 2016 *K. pneumoniae* becomes hyperendemic, driven by an increase in neonatal infections. Genomic data show repeated waves of clonal expansion of different, often ward-restricted, lineages, suggestive of hospital-associated transmission. We describe temporal trends in resistance and surface antigens, of relevance for vaccine development.

Conclusions Our data highlight a clear need for new interventions to prevent rather than treat *K. pneumoniae* infections in our setting. Whilst one option may be a vaccine, the majority of cases could be avoided by an increased focus on and investment in infection prevention and control measures, which would reduce all healthcare-associated infections and not just one.

*Correspondence:

Eva Heinz

eva.heinz@lstmed.ac.uk

Nicholas A. Feasey

Nicholas.feasey@lstmed.ac.uk

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Keywords AMR, Malawi, Genome surveillance, Healthcare-associated, Nosocomial infections, Surface antigens, Neonatal infection, Sepsis, Capsular polysaccharide, ESKAPE

Background

The burden of deaths in children under the age of five, including those due to neonatal sepsis, is especially high in low- and middle-income countries (LMICs) [1]; and one of the main pathogens causing these is *Klebsiella pneumoniae* (*Kpn*) [2]. Until the last decade, *Kpn* was typically readily treatable with beta-lactam antimicrobials, in particular third-generation cephalosporins (3GC). The effectiveness of 3GC is lost when these bacteria acquire extended-spectrum beta-lactamase enzymes (ESBLs). For *Kpn* there has been a drastic increase in 3GC-resistance by acquisition of ESBL enzyme-encoding plasmids; the proportion of *Kpn* hospital isolates that are ESBL-producing increased from 12% to > 90% in parts of sub-Saharan Africa (sSA) in the last decade [3]. The main remaining treatment option is carbapenems; these however are uncommonly available in sSA, rendering *Kpn* effectively untreatable in large parts of Africa. This is particularly problematic for neonatal sepsis caused by *Kpn*, and there is increasing evidence from large studies such as BARNARDS [2] and CHAMPS [4] that the burden of disease caused by *Kpn* falls most heavily on this patient group. Treatment options for neonates are even more limited than for other age groups as therapy needs to be intravenous and some antimicrobial classes are contraindicated, however, delay in establishing appropriate therapy is even more dangerous.

The population structure of *Kpn*, similar to other opportunistic pathogens that thrive in the environment, is highly complex [5]. Understanding long-term population trends of clinically relevant lineages at both a local and global scale, and which antimicrobial resistance plasmids and elements they encode, is potentially relevant to guiding treatment, infection prevention strategies and vaccine development. However, few longitudinal studies of the *Kpn* population have been published so far, and the majority have come from collections established in high-income settings or South East Asia [6–10], whilst data from sub-Saharan Africa, South America and the Middle East and North Africa (MENA) remain sparse. Low-income countries in sub-Saharan Africa are particularly underrepresented in ‘global’ collections of pathogen genome data, despite often carrying the highest burden of disease [2].

Whilst *Kpn* has a global distribution, there are clear geographical concentrations of dominant lineages and resistance genes, likely reflecting local antimicrobial pressure, patient populations in particular regarding

comorbidities like HIV prevalence or malnutrition, and a range of other determinants. Developing a more representative picture of the global epidemiology of *Kpn* is relevant to the development of vaccines targeting *Kpn*, to ensure that the lineages and associated LPS O-antigens and capsular polysaccharide antigens most prevalent in countries with the highest burden of disease are targeted. A better understanding of the genomic epidemiology of *Kpn* in low-income countries is also crucial to advocate for access to antimicrobials, in particular newer agents, which is often profoundly limited by cost, leaving *Kpn* infections that can be readily treated in high- or middle-income countries de facto untreatable. Further, knowing at a facility level where problematic hotspots of transmission are within a hospital setting can help focus infection prevention and control (IPC) efforts.

To address this urgent need for a better understanding of the *Kpn* population in sSA, we performed whole-genome sequencing on all bloodstream isolates phenotypically identified as *Kpn* from Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, which has undertaken sentinel surveillance of bacteraemia and meningitis in partnership with the Malawi-Liverpool Wellcome Programme (MLW) since 1998 [3]. Our analyses include all isolates, irrespective of resistance profile or patient demographic, from 1996 to 2020, with no change in sampling strategy from 2000 to 2020. We report the shifts in resistance profile and population structure over time and highlight the relevance to consider trends over time and not just total numbers for considerations of new drug regimens or vaccine target selection.

Methods

Isolate collection

QECH is a government-run tertiary referral centre for the southern region of Malawi, which provides free care at the point of delivery. It serves a population of 800,000 for the area of urban Blantyre (Malawi census 2018). There are adult and paediatric A&Es, medical and surgical wards. There is a tertiary neonatal unit on-site and a separate paediatric surgical hospital which opened in 2017 (Fig. 2C; PICU, Paediatric HDU, Paediatric surgical ward).

Microbiological sampling and processing

Routine, (ISO15189 accredited since 2019) diagnostic blood culture services have been provided to the medical and paediatric wards by the Malawi-Liverpool-Wellcome

Programme since 2000. For adults 7–10 mL of blood were collected from all patients admitted to the hospital with fever (axillary temperature >37.5 °C) or clinical suspicion of sepsis, severe sepsis, or septic shock. Sepsis, severe sepsis, or septic shock were suspected in patients with tachycardia (≥ 90 beats per minute), hypotension (systolic blood pressure <90 mm Hg), tachypnoea (respiratory rate >20 per minute), or delirium. Three to 10 mL of blood was taken from children with non-focal febrile illness who tested negative for malaria, who were severely ill with suspected sepsis, or who failed initial malaria treatment and remained febrile. For adults and children with clinical suspicion of meningitis (temperature >37.5 °C with seizures, headache, abnormal behaviour or meningism), a lumbar puncture was also performed, and the sample was sent for CSF analysis. For neonates with clinical signs of sepsis or meningitis (temperature >37.5 °C, or other signs of clinical deterioration such as reduced activity, increasing work of breathing or respiratory rate >60 , heart rate >180 or <100 , and unexplained seizures) a blood culture and CSF sample was collected. Afebrile patients were unlikely to have had blood or CSF cultures unless the clinical suspicion for sepsis or meningitis was high.

For blood cultures, samples were collected using aseptic methods and inoculated into a single aerobic bottle (BacT/Alert, bioMérieux, Marcy-L'Etoile, France). These were incubated using the automated BacT/Alert system (bioMérieux, France) since 2000. Before which, they were cultured manually. Samples that flagged positive were Gram-stained and Gram-negative bacilli are identified by the Analytical Profile Index (Biomérieux). CSF samples were processed for cell count and biochemistry before manual culture. Antimicrobial susceptibility testing was determined by the disc diffusion method (Oxoid, UK) using AST breakpoints that were current at the time, initially following the relevant version of the British Society of Antimicrobial Chemotherapy's guidelines until 2018, after which time EUCAST methods and breakpoints were introduced. Organisms which showed reduced inhibition when exposed to ceftriaxone 30 mg discs, ceftriaxone 5 mg discs, or cefpodoxime 10 mg discs were classified as resistant to ceftriaxone. Intermediate isolates were classified as resistant to ceftriaxone. Details for the identification of other organisms are described elsewhere [3]. *Klebsiella pneumoniae* isolates were stored at -80 °C on microbank beads.

Whole genome sequencing and QC

All *Klebsiella pneumoniae* isolates that could be recovered from the start of the MLW archive to April 2020 were thawed and incubated on MacConkey's media for 18–24 h at 37 °C. Plates with pure growth then had

a single colony pick taken and inoculated into 15 ml of buffered peptone water for 18–24 h at 37 °C. These samples were then centrifuged and the supernatant was discarded. The pellet was then resuspended in buffer and the DNA was extracted using the QIA Symphony machine and QIA Symphony DSP kit with onboard lysis, according to the manufacturer's instructions. Quality control was done using Qubit and samples with a DNA volume of less than 200 ng were repeated. DNA was sequenced at the Wellcome Sanger Institute on the Illumina HiSeq X10 instrument (Illumina Inc., USA) to produce 150-bp paired-end reads.

Eleven out of 1313 samples failed sequencing; six of these were below the Sanger-internal threshold for minimum DNA amount (0.2 µg total); these eleven samples were removed from further analyses. Quality control on all remaining isolates was performed using species confirmation via Kraken v0.10.6 [11] to identify contaminants (incorrect species). Any species assignment other than *Klebsiella pneumoniae*, *Klebsiella variicola*, unclassified and synthetic construct were considered contaminants; the latter two categories frequently derive from plasmid sequences in our experience. All isolates with higher than 10% of other species were removed as contaminated (Fig. S2A). Similarly, isolates with a ratio of heterozygous SNPs over 5% were removed as likely contaminated by a related strain (Fig. S2B). We note that four sequence runs; 42092_2#162, 42070_2#207, 42092_2#170, 42092_2#237; include a higher proportion of 'unclassified' reads, whose origin is unlikely from a bacterial contaminant but might be from phages, unknown plasmid sequences, or other unclassified components, leading to larger than usual assembly size (>6 Mb; Table S1). Further quality control used the assemblies, where all isolates resulting in more than 500 contigs were removed as likely contaminated by other species or strains, or of very low quality (Fig. S2C). De novo assembly of the remaining 1156 genome sequences was performed using SPAdes v3.14.0 [12] incorporated into the bacterial genome assembly pipeline described previously [13]. Annotation of all assemblies was performed using prokka [14] v1.14.5.

Long-read sequencing and hybrid assemblies

To quality-control our short-read DNA extraction, three selected strains, representing major sequence types and complex plasmid combinations, were used for long-read sequencing. DNA was isolated using the MasterPure Complete DNA and RNA isolation kit to retrieve long fragments, and sequencing was performed using the Oxford Nanopore MinION platform with R9 flow cells. Base calling was performed using guppy (v6.5.7, <https://community.nanoporetech.com>), and the initial assembly was performed with flye (v2.9.2, [15]). The assemblies

were then polished with medaka (v1.8.0, <https://github.com/nanoporetech/medaka>) which uses the long read sequence data followed by polypolish (v0.5.0, [16]) including the insert size filter step as recommended by the author. The assemblies were then annotated using prokka as above, and plasmid replicons in these assemblies were determined by submitting to plasmidfinder [17] via the online platform (<https://cge.food.dtu.dk/services/PlasmidFinder/>; software version 2.0.1; database version 2023–01–18). Accession numbers and contig details are provided in Table S6.

Genome analyses and phylogenetics

For comparative analyses, isolates from recent studies at QECH were included, as they are part of the bloodstream collection [18–20]. Details of all sequences used in the analyses are given in Supplementary Table 1. Resistance gene prediction and plasmid replicon types were performed using ariba [21] (v2.14.6) and the srst2-argannot [22, 23], CARD [24] and plasmidfinder [17] databases (download date 14.02.2022). To assess aminoglycoside resistance; ANT3 is grouped by ariba as AacAad_AGly, AadA4_5_AGly, AadA_AGly, AadA_AGly_2, AphA6_AGly; AAC(3)-II is grouped by ariba as Aac3_Ia_AGly; ANT(2'')-I is grouped by ariba as AadB_AGly and AAC(6')-I is grouped by ariba as AacAad_AGly_3.

Prediction of sequence types, (sub)species and *Klebsiella*-specific virulence factors were performed using kleborate v2.1.0 [25] and the `-r` setting; the results are given for all isolates in Table S1. O- and K-types were predicted with kaptive [26] (v2.0.6) which incorporates O-loci as well as O-serotype prediction [26–28]; and we added an additional option of *wbbZ* as extra gene to distinguish between O1a (O1/O2+*wbbY*, no *wbbZ*) and O1b (O1/O2+*wbbY*+*wbbZ*) as described recently [29], adding *wbbZ* as implemented in kaptive versions before v2 [27] and expanding the .logic file to distinguish O1a/b. As the *wbbZ* gene is located outside the O-locus, we additionally searched for this gene (reference sequence LT174607.1:7925–8734) using ariba as described above.

Core genome SNPs for *K. pneumoniae* subsp. *pneumoniae* isolates were determined using snippy [30] (v4.6.0; <https://github.com/tseemann/snippy>) with the *Klebsiella pneumoniae* subsp. *pneumoniae* NTUH-K2044 genome (AP006725.1) [31] as reference. Recombinant regions were removed using gubbins [32] (v3.2.1) and the Fast-Tree [33] option ('-tree-builder fasttree'), given the very high number of samples, as implemented in gubbins; and only isolates from sequence types with more than one isolate in our dataset were included in the analysis to further reduce compute time. The same strategy was used for *K. quasipneumoniae* and *K. variicola* using strains ATCC 700603 (CP029597.1) [34] and At-22

(CP001891.1) [35] as reference, respectively, where all isolates were included and default options for gubbins were used (RaxML for tree generation, given the much smaller sample numbers). A snp-only alignment was generated from the recombination-free gubbins output using snp-sites [36] v2.5.1 with the `-c` option (selecting for ACGT-only) for *K. quasipneumoniae* and *K. variicola*; for *K. pneumoniae* subsp. *pneumoniae*, given the high variation of isolates, the `-c` option was too stringent (resulting in a < 200-bp alignment) and we thus used snp-sites to isolate all snps but without selecting for ACGT-only sites.

The snp-only alignments were used as input for iqtree [37] (v1.6.12), the number of constant sites was determined with snp-sites `-C` function from the original full alignment and provided to the '`-fconst x,x,x,x`' iqtree function. The model was determined with the integrated ModelFinder [38] function '`-m TEST`' resulting in GTR+F+I+G4, GTR+F+I+G4, TVM+F+I+G4, for the alignments from *K. pneumoniae*, *K. quasipneumoniae* and *K. variicola*, resp. We set '`-keep-ident`' to keep identical data and '`-bb 1000`' for 1000 ultrafast bootstrap replicates. The resulting trees were then adjusted to SNP-length branch lengths using the python implementation of pyjar [39] (<https://github.com/simonrharris/pyjar>) as input for rPinecone [40]. Closely related clusters taking their phylogenetic distance into account were determined with 5 SNPs as cut-off for minor and 10 as cut-off for major clusters. These were visualised with iTol [41] (v6.8) with visualisation files generated as implemented in rPinecone [40]. For ST35, ST39 and ST14/15 (combining ST14 and ST15 isolates in one analysis) we performed ST-specific mappings of all respective isolates against references from these sequence types to gain higher resolution (CP041353.2 [42], LR991401.1 [43] and CP008929.1 [44], resp.).

Pan-genome reconstruction, generation of a core gene alignment, and identification of neighbouring gene regions were derived via panaroo [45] (v1.3.3) using the '`-clean-mode strict`' setting. The phylogenetic tree based on the core gene alignment, with informative sites extracted with snp-sites as above, was calculated using iqtree [37] (v1.6.12) and the internal ModelFinder [38] function (GTR+F+ASC+G4) as above. All other metadata summary analyses and visualisation were generated using the ggplot [46] and ggtree [47] packages in R [48] unless indicated otherwise; some colour schemes are derived from or inspired by the MetBrewer (<https://github.com/BlakeRMills/MetBrewer>) and National-Parks (<https://github.com/katiejolly/nationalparkcolors>) R libraries. Composite figures were generated using the patchwork package (<https://github.com/thomasp85/patchwork>). An RMarkdown file to generate the analysis plots and the underlying tree and data files are freely

available online (<https://github.com/EvitaH/QECHospitalKlebs>; <https://doi.org/10.5281/zenodo.8421658>).

Results

The QECH bloodstream isolate collection

QECH is the main referral hospital for the southern region of Malawi and the main hospital for Blantyre, the second largest city of Malawi (population 800,024 at the 2018 census). It has approximately 1000 beds and receives around 10,000 adult medical and 30,000 paediatric medical admissions per year. A quality-assured diagnostic microbiology service was initially implemented in partnership with MLW at QECH in 1998. Laboratory blood culture was initially manual, and an automated system was implemented in 2000 (BacT/ALERT, Biomerieux). The diagnostic microbiology service expanded to culture cerebrospinal fluid (CSF) from 2002. During the study period, all adult and paediatric medical patients with suspected sepsis or meningitis were eligible for blood and/or CSF culture at the discretion of the admitting physician, and where possible before the administration of antimicrobials. Bacterial isolates were identified by standard techniques detailed in the methods.

At the start of the period, the first-line treatment against bloodstream infections was crystal-penicillin and chloramphenicol although gentamicin was used in neonates instead of chloramphenicol. This began to change with the introduction of the 3rd-generation cephalosporin ceftriaxone from 2004, which was rapidly adopted as a first-line agent by the Department of [adult] Medicine and more slowly by the Department of Paediatrics, which continues to use penicillin and gentamicin in the management of neonatal sepsis, as per WHO guidelines [49].

Members of the order Enterobacterales including *Kpn* were identified by API biochemical index (Biomerieux, France). *Klebsiella pneumoniae* is known to comprise a species complex including *Klebsiella pneumoniae* subsp. *pneumoniae* as well as several closely related species like *K. variicola* and *K. quasipneumoniae* and their respective subspecies [50]. These can only be identified to species complex level via API, and we therefore refer to all members of the *K. pneumoniae* species complex as *Kpn* and specify when referring to specific species (Fig. 1A).

Dramatic increase in *Kpn* cases from 2016

The crude frequency of *Kpn* cases was high at the start of the period with 121 cases in 1998, although this overlapped with a year of active surveillance of febrile adult patients [51] and we thus focused on longitudinal analyses from 2000 onwards. The number of cases from 2000 to 2015 was stable with a mean of 44 cases per year

(Fig. 1A). This increased to a mean of 187 cases per year from 2016 to 2021 with a peak of 279 in 2019.

Neonatal infection with *Kpn* accounted for 39.3% of all *Kpn* infections, even though only 11.2% of all blood cultures were taken from this patient group. The overall rate of *Kpn* isolation was 24.9 per 1000 blood cultures for neonates, 4.3 per 1000 blood cultures for other patients (rate ratio of neonates vs non-neonates 5.11 (95% CI 4.66–5.61)). The increase in *Kpn* cases post 2016 was driven by an increase in neonatal infections (Fig. S1A–1G); there were peaks in the *Kpn* blood culture isolation rate in neonates in 2008, 2014 and 2017 which suggest outbreaks or ongoing transmission on the neonatal unit (Fig. 1B), and our genomic analyses further support hospital transmission given the close genetic relationships of the respective isolates. Though the blood culture positivity rate was increased for most age groups in the period from 2016 to 2021, for neonates this increase was particularly marked (33.5 per 1000 blood cultures in the period 2016–2021 compared to 15.4 per 1000 blood cultures in the period 1998–2015; Fig. S1A–1D). As well as the peak in neonatal cases, there was also a much smaller peak in the adult age group, which shifted from the 30–35 year age group in the period from 1998 to 2015 to the 35–40 year age group in the period from 2016 to 2021 (Fig. S1E–1H).

Increase in *Kpn* numbers driven by temporally defined expansions of sequence types (STs)

We retrieved 1313 isolates not previously sequenced from the QECH *Kpn* collection, 1302 of which could be recovered for growth and yielded enough DNA for sequencing; and 1156 of these passed all quality control steps for further analysis (for details, see the ‘Methods’ section; Fig. S2). In addition, we included 217 samples from previously published studies of this collection [18–20], resulting in 1373 sequenced genomes analysed in this study (Fig. 1C).

The majority (1274/1373, 92.8%) belong to *Klebsiella pneumoniae* subsp. *pneumoniae* with only very small proportions representing other (sub)species, including *K. quasipneumoniae* subsp. *quasipneumoniae* (10/1373, 0.7%), *K. quasipneumoniae* subsp. *simillipneumoniae* (46/1373, 3.4%) and *K. variicola* subsp. *variicola* (43/1373, 3.1%). Based on phenotypic microbiology data (Fig. 1A and B) we observed a drastic increase in isolates from neonatal infection in 2016/2017 which is reflected in our sequenced collection.

Assessing the diversity of STs over time highlights that the increase in *Kpn* infections represents repeated increase and subsequent decrease in numbers of different multilocus sequence types (Fig. 2A). Whilst the earlier years show a diverse composition of STs with small numbers of each ($n < 15$ total), the increase in total *Kpn*

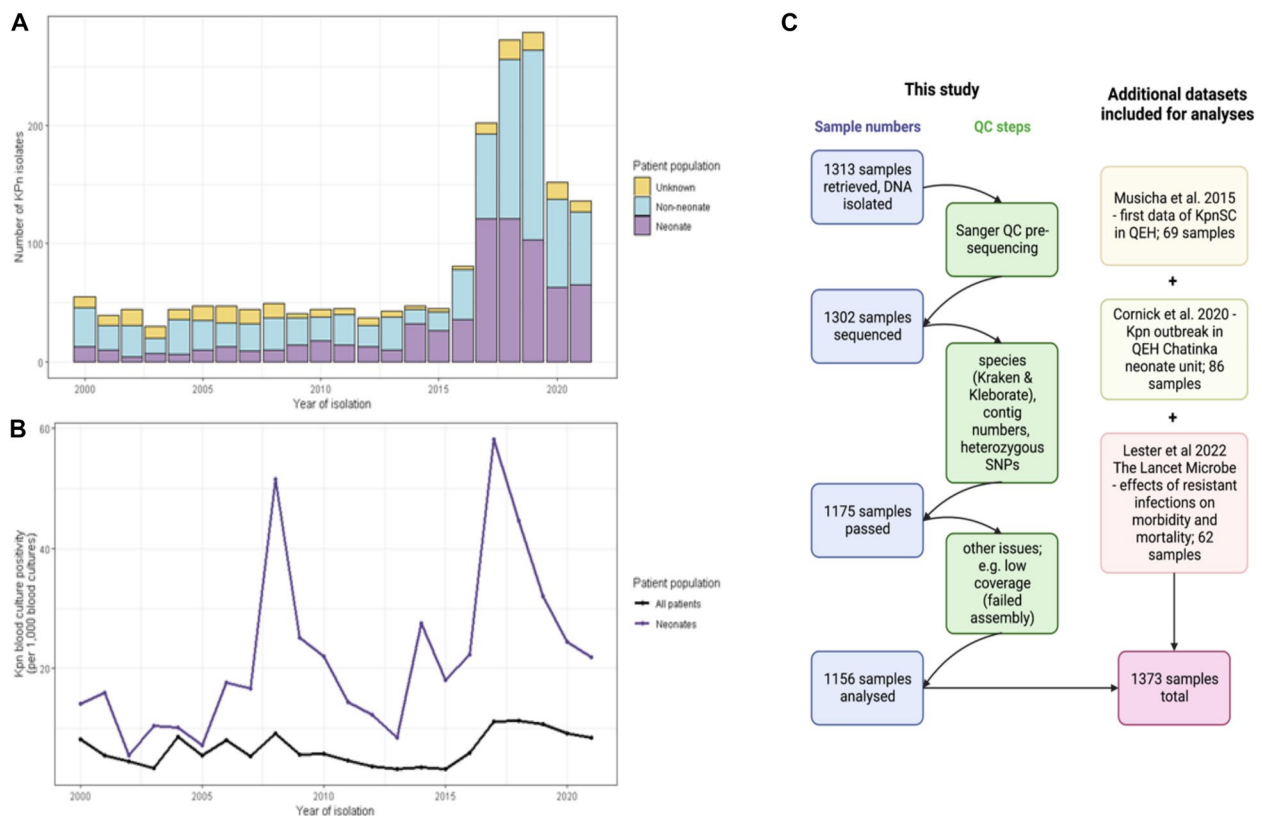


Fig. 1 *Kpn* cases at QECH. **A** Numbers of *Kpn* cases per year at QECH. Bars represent the crude frequency of *Kpn* infection for each year from 1998 to 2021, with the different colours representing the different age groups of the patients. **B** Blood culture positivity rate (per 1000 blood cultures) of *Kpn* in neonates and the entire patient population. **C** A diagrammatic overview of the samples included in this study (created with www.biorender.com)

numbers is mainly driven by one or two major STs, with these peaks spanning approximately 1.5 to 2 years each. We furthermore observe that after the increase of isolates of the major STs, each of them reverts to their original very low numbers (Fig. 2A).

A previous study [19] highlighted an outbreak of ST340 across the neonatal unit which is reflected here, where it comprises 31 out of 105 isolates in 2013 and 2014 (29.5%; Fig. 2A). We then see an increase of the numbers of ST14 (19/44 in 2015; 43.2%) which is present at a lower proportion before and after this period; followed by an increase in ST25 (13/87 in 2016; 14.9%) which again is present at lower proportions before and after. The year 2017 then saw a steep increase in *Kpn* infections overall; mainly driven by an expansion of ST39 which comprises more than half the samples at the time (100/188 in 2017; 53.2%). This clone however again retreats back to low numbers, with a few lineages dominating, in particular, ST1552 (35/208 in 2018; 16.8%) and ST35 (40/208 in 2018; 19.2%). The year 2019 then sees another increase in ST14 (61/236 in 2019; 25.8%) as well as ST15 (30/236 in 2019; 12.7%), which is a known high-risk clone

predominantly found in South East Asian collections where it has been linked to an early spread of carbapenem resistances via *bla*_{NDM} genes [52, 53]. Interestingly, ST307, a major resistance clone highlighted in other parts of the world [54], only formed a small proportion with only a single year of over 10% (3/28 in 2013; 10.2%).

Isolation of *Kpn* from neonatal and paediatric wards account for observed changes, whilst adult infections remain stable over time

The patterns over time differ significantly between wards (Fig. 2B), suggesting the increase in *Kpn* infections could represent a pattern of repeated ward-specific outbreaks, rather than a generalised increase across all wards (Fig. 2B). This pattern of ward-focused increases in isolate numbers was repeatedly observed on the Chatinkha neonatal unit and the surgical PICU/HDU, where most of the changes in numbers of isolates occur. An indication of local outbreaks is further supported by analysing the major STs, which increase at defined times in specific wards (Fig. 2B and C; Fig. S3). To investigate the impact of closely related isolates (<5 core SNP distance) that

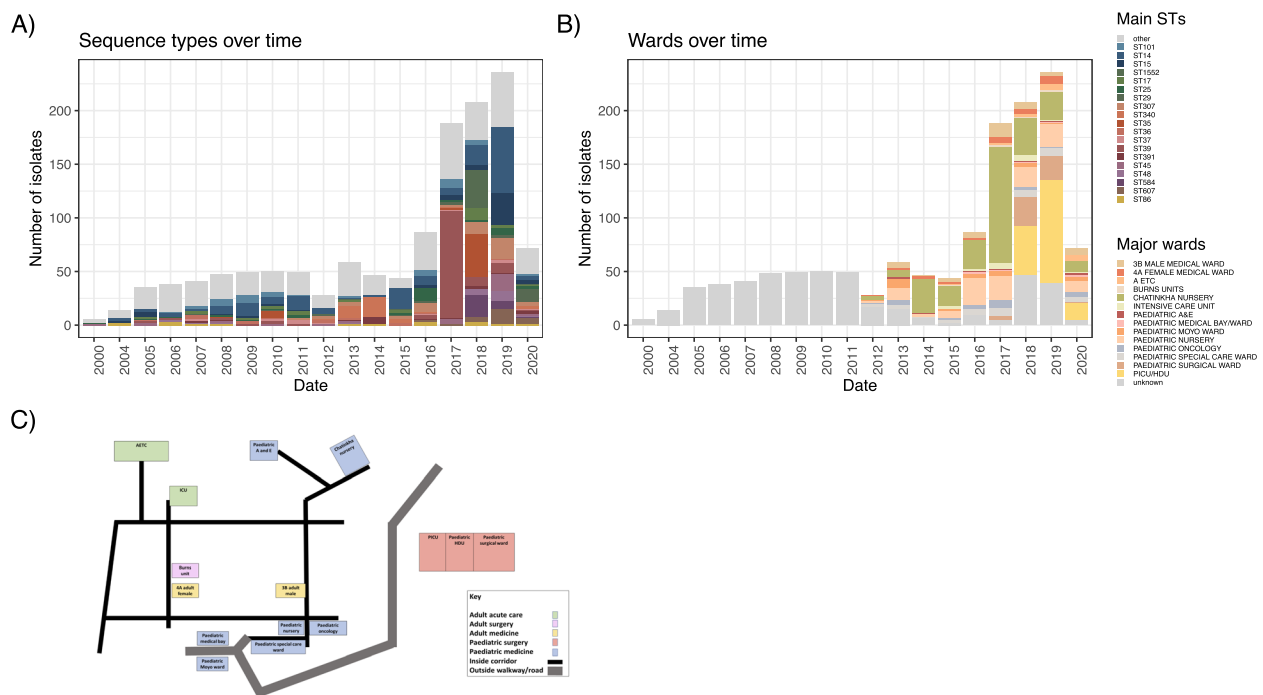


Fig. 2 Uneven distribution of STs and cases at QECH wards indicate hospital-acquired infections. **A** Main STs and **B** wards the samples were derived from over time. **C** A schematic map of QECH with the wards samples of our study were derived from highlighted. AETC, Adult emergency and Trauma Centre; Paediatric A&E, paediatric accident and emergency; PICU, paediatric intensive care unit; HDU, high dependency unit

are likely within-hospital transmissions on the number of infections overall, we analysed a core genome tree for each of the three species and identified closest-neighbour SNP distances in a root-to-tip approach [40] (Fig. 3). This highlighted that most highly clonal lineages (defined as <5 SNPs) are derived from *K. pneumoniae* subsp. *pneumoniae* (Fig. 3A) representing 402 of 1165 isolates as a conservative estimate based on whole-species analyses, with 43% of the *K. quasipneumoniae* subsp. *similipneumoniae* (20 of the 46 isolates in 6 lineages, Fig. 3B) and 25% of the *K. variicola* isolates (10 of the 40 isolates of this species, Fig. 3C); and no <5 SNP-related isolates of *K. quasipneumoniae* subsp. *quasipneumoniae*.

Despite removing the single-isolate STs for this analysis, our dataset still contains a high number of *K. pneumoniae* subsp. *pneumoniae* STs, which results in a highly diverse dataset. As this diversity has potential to impact on tree resolution depending on the reference used [55], we investigated three key STs in more detail. We focused on ST35, ST39, and the closely related ST14 and 15 together, whose pattern of accumulation in a ward and year strongly indicates multiple hospital-associated transmissions (Fig. S3), in more detail by mapping against strain-specific reference genomes (Fig. 3A). ST39 and ST35 are two key STs when considering the per-year breakdown of isolates per ward for the neonatal unit

(Chatinka; Fig. S3); whilst ST14 and ST15 were identified in high numbers in the PICU/HDU in the paediatric surgical ward (Fig. S3). Here, we can observe that 77% of the isolates (51/55 ST35, 110/136 ST39, 182/254 ST14-15) have their nearest neighbour within the threshold of 5 SNPs; representing 100% (17/17) of ST35 and 88.9% (80/90) of ST39 from Chatinka and 88% (57/65) ST14 and 15 from the PICU/HDU (Fig. 3A). This high relatedness of isolates strongly supports within ward transmission and the argument that *Kpn* is a healthcare-associated cause of neonatal sepsis. Whilst details like length of stay and within-ward location are not available, the fact that patients are regularly transferred from Chatinka to the PICU/HDU if surgical procedure is required means that these wards are already linked by a clear transmission path via patients, and several potential between-ward transmission events are indicated in particular in ST35 and ST14/15 (Fig. 3D, F).

Over the period from 2015 to 2018, there was an increased focus on performing blood cultures to ensure every neonate on performing blood cultures to ensure every neonate on Chatinka nursery that met the clinical criteria for suspected neonatal sepsis was included, which may explain some of the large increase in *Kpn* cases in 2017 on the nursery. Establishing optimal infection prevention and control (IPC) has been an ongoing challenge [56]; specifically, handwashing has been difficult due to

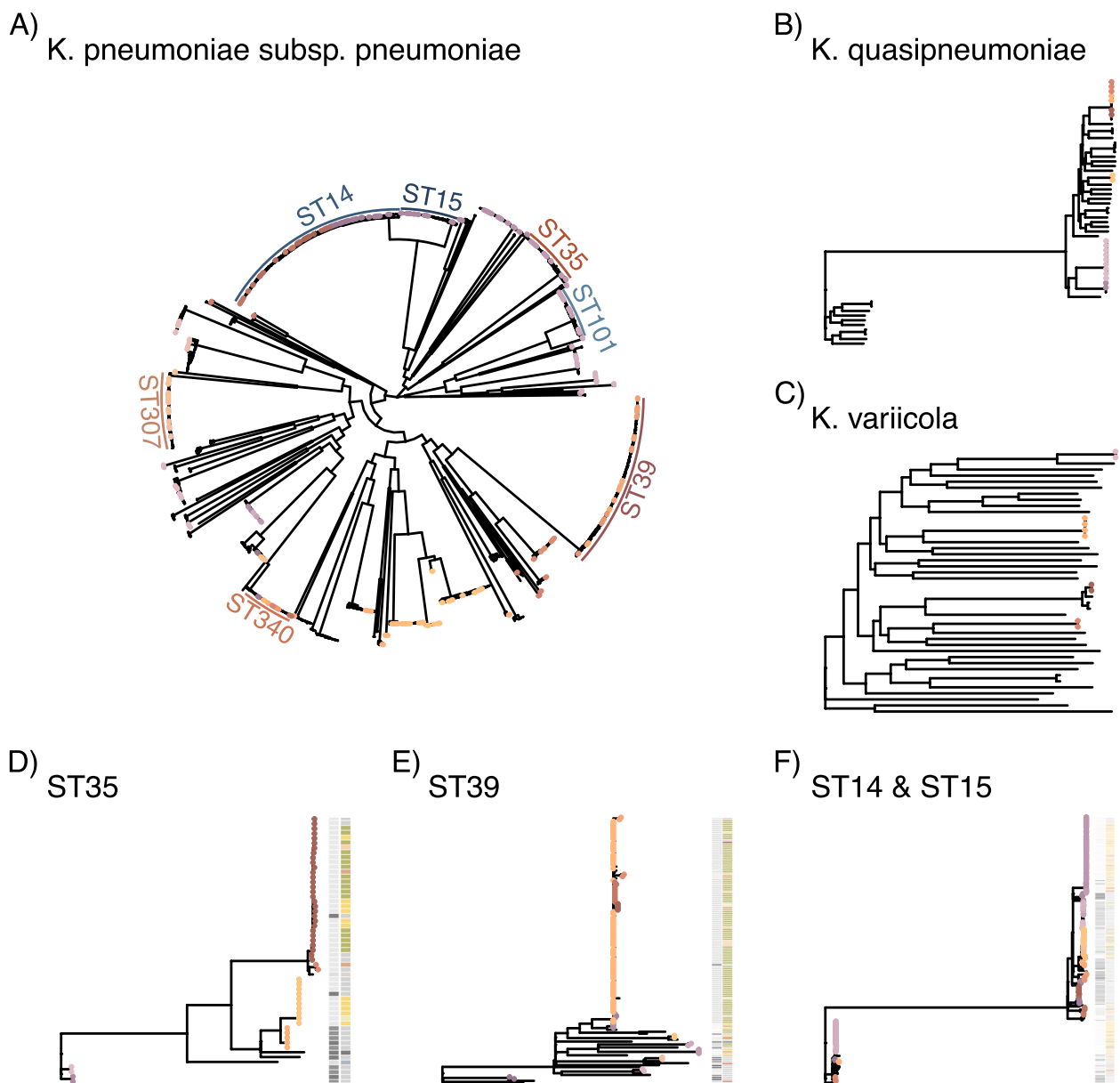


Fig. 3 Most cases suspected to be caused by within-ward transmissions. Mapping trees against reference genomes for **A** *K. pneumoniae* subsp. *pneumoniae* excluding STs represented with only a single isolate in the dataset, **B** *K. quasipneumoniae* and **C** *K. variicola*. **D** and **E** show ST-specific mapping of the relevant isolates against references for ST35 (**D**), ST39 (**E**), and ST14/ST15 (**F**). The coloured tips indicate isolates with <5 SNPs distance to their nearest neighbour based on a root-to-tip approach to identify related clusters of isolates [40]. The colour ring in **A** shows the main STs, the columns in **D–F** show the year of isolate (left column) and major ward (right column)

intermittent water supply and a lack of access to soap or hand sanitiser and there are limited numbers of cots, so neonates have often shared them. In response to the large increase in *Kpn* cases, there was a renewed focus on IPC, hand sanitiser and soap procurement was prioritised, deep cleans of the ward environment were performed regularly, there was increased cleaning of ward surfaces, ward traffic was limited, wooden cots are being replaced

with easier to clean plastic ones, the number of neonates sharing a cot was reduced and oxygen delivery devices are being replaced with single-use items.

Antimicrobial resistance and implications for treatment

Phenotypic resistance profiles of the strains, where available, mirror the very concerning trends reported previously for *Kpn* in QECH [3] (Fig. S4, Table S4). Resistance

against the frontline treatments is widespread; a particular concern is a high proportion of isolates with phenotypic resistance to ceftriaxone (83.6% in 2019), gentamicin (79.3% in 2019) and cotrimoxazole (93.0% in 2019), which rapidly emerged from 2012 onwards with very little change over time (Fig. S4A–C; >75% of tested isolates were resistant to these agents each year 2012–2020). We notice some variation in co-amoxiclav (augmentin: amoxicillin + clavulanic acid) susceptibility profiles but from 2019 again show >75% of tested isolates as resistant (Fig. S4A). Importantly, we notice that a trend of increased numbers of chloramphenicol susceptible isolates (Fig. 4B, Fig. S4G), which has previously reported in Malawi [3], is also explicit in our *Kpn* collection in particular in recent isolates (38.7%, 51.5%, 72.2% isolates sensitive for 2018–2020). The presence of *catA1* or *catA2* agrees well with phenotypic resistance whereas the presence of *catB4* did not as shown in Fig. 4B; where we see a large number of isolates with sensitive phenotype, no *catA1/2* but *catB4*, whereas only a small fraction of isolates has resistant phenotype, no *catA1/2* but *catB4* where we hypothesise a different mechanism is likely driving the resistant phenotype.

For isolates with resistant phenotype and encoding both *catA1/2* and *catB4*, the resistance is presumed to be driven by *catA1/2*. This agrees with *catB4* representing a non-functional truncated variant of *catB3* as was recently reported [57]. We furthermore note a sensitivity of 50% or higher in the majority of years measured in ciprofloxacin (6/9 years from 2012 to 2020, including 2017, 2018 and 2020; Fig. S4E, Table S4), which together with chloramphenicol thus represent two antimicrobials that might thus be valid treatment options.

Resistance to 3GC was widespread (83% resistance to cefpodoxime/ceftriaxone by 2020; Fig. S4A) and almost exclusively driven by the acquisition of the *bla*_{CTX-M-15} gene, which continuously outcompetes other ESBL-encoding genes that are occasionally present in the *Kpn* population, but which are only sporadically detected (Fig. 4A, Table S2). The number of plasmid replicons per isolate stabilised after the first increase in numbers around 2015, highlighting that a few large AMR plasmids are likely responsible for the majority of AMR-isolates (Fig. S5C, Fig. S6B, FigS7B, Fig. S8A, Table S3). Strains with a moderate number of plasmid replicons dominate the population (0–5) and this remains stable over time.

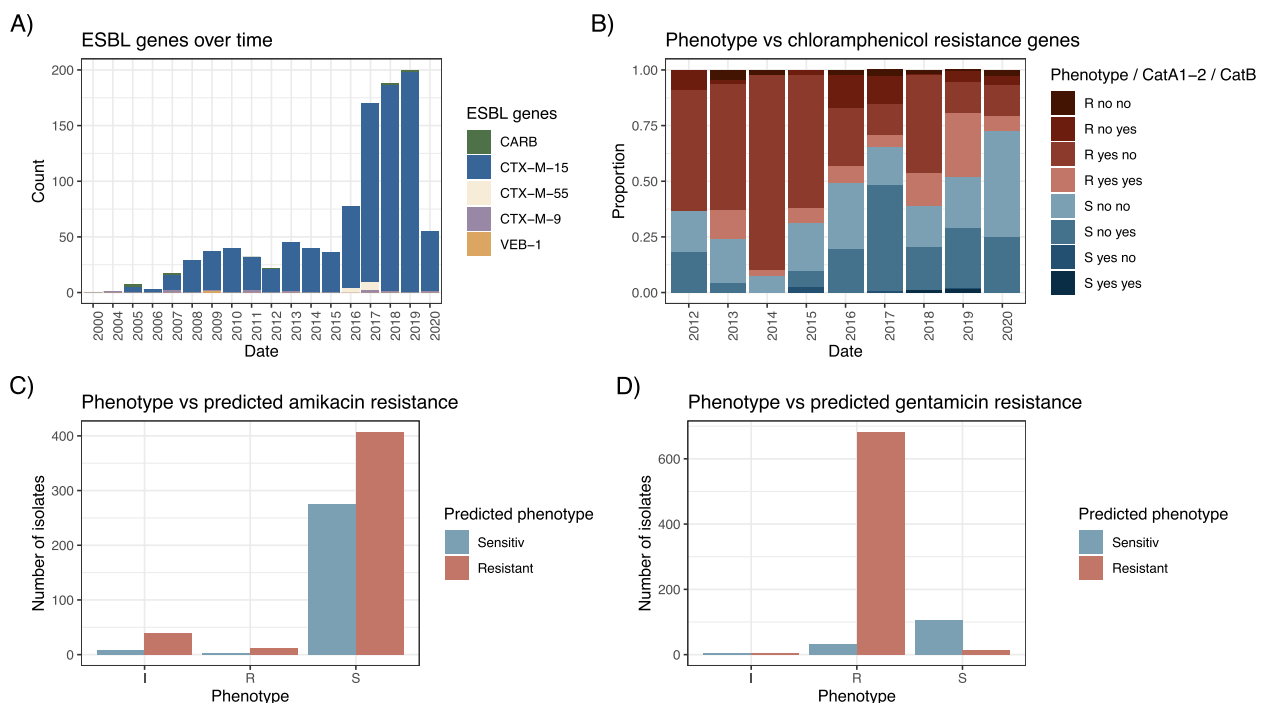


Fig. 4 Resistance mechanisms only explain beta-lactam, not aminoglycoside, phenotypes. **A** The distribution of ESBL genes over time illustrating the dominance of *bla*_{CTX-M-15}. **B** Phenotypic chloramphenicol resistance profiles proportional over time and the presence or absence of *catA1* and/or *catA2* compared to *catB4*, the recently re-characterised non-functional CAT enzyme. **C** and **D** Predicted genotypic aminoglycoside resistance was based on the presence of an *Aac6'-Ib-cr* or *Aac6'-Ib* gene (*AAC(6')-I*) for amikacin and *Aac3_Ila* (*AAC [3]-II*) or *AadB* (*ANT(2'')-I*) for gentamicin. The x-axis shows the samples based on phenotypic resistance profiles (intermediate, sensitive, resistant) against **C** amikacin and **D** gentamicin, and the bars show the number of isolates in the respective category that would have been predicted as sensitive (blue) or resistant (red) based on the genes as above

Only a small number of isolates with more than five plasmid replicons associated was identified at any point in time over the whole time period investigated (Fig. S8); and using long-read sequencing on two selected isolates from major STs confirms the predicted low number of plasmid replicons and thus also plasmids (Fig. S9), again highlighting that the repertoire of resistance plasmids that led to the success of *Kpn* in this setting was driven by the change to a predominantly ESBL⁺ population as the key event, with the population then remaining stable over time with respect to plasmid diversity and numbers of resistance genes (Fig. S8B).

Carbapenem resistance genes are still only rarely observed; we identified two samples encoding for *bla*_{NDM-1}; one in a ST39 in 2016 and one in a ST45 in 2019, perhaps unsurprisingly as carbapenem usage is still comparatively unusual. We also investigated resistances against other potential alternative treatment options colistin, fosfomycin and tigecycline. Resistance to these agents is all at low levels, with only one fosfomycin resistance gene detected (*fosA*) and none against colistin or tigecycline predicted in our dataset, and one isolate with predicted mutations that can confer resistance to colistin (*pmrB*), indicating that these might be viable alternatives for ESBL⁺ infections at QECH, although tigecycline is contraindicated in children under 8y (Table S2) and neither agent ideal treatments for bacteraemia [58, 59]. Tigecycline resistance is often conferred by upregulation of transport proteins and thus challenging to predict and has also been observed during within-patient evolution [60–62]; we could however not detect the described plasmid-derived antimicrobial resistance gene *tetX* in our collection [63].

Based on the AMR phenotype identified by disc diffusion, amikacin remains a viable alternative to gentamicin, although there are concerns over blood–brain barrier penetration in cases of meningitis and renal toxicity in settings where therapeutic drug monitoring is not possible. Phenotypic data are corroborated by a more detailed analysis of the aminoglycoside AMR genes (Fig. 4C and D). In total 90.5% of all isolates (1243/1373) are predicted to encode one or more aminoglycoside resistance genes, however, the majority are predicted to confer resistance to streptomycin (ANT3) and gentamicin (AAC(3)-II; ANT(2'')-I). Figure 4D highlights these as ‘predicted resistant’ (ANT3 for streptomycin, AAC(3)-II and/or ANT(2'')-I for gentamicin). Only a fraction of isolates (43.2%, 593/1373) encoded AAC(6')-I; which can provide resistance against amikacin; the presence of this gene is interpreted as ‘predicted resistant’ in Fig. 4C. Aminoglycoside resistance is known to be challenging to predict [64]; whilst phenotypic gentamicin resistance testing matches very well with the predicted profile based on

acquired genes, the presence of AAC(6')-I does not by itself confer resistance in a large number of isolates, with only 12 isolates encoding for either of these, representing 2%, actually showed a resistant phenotype (Fig. 4D; Table S4).

Potential vaccine target stability

The *Kpn* polysaccharide antigens are of high interest as vaccine targets; in particular the large capsular antigen. As expected, we observed a very high number of different K-loci (90 predicted), as well as a diverse set of O-loci (15 predicted), the latter are known to be far less diverse in *Kpn* [28]. Recent findings furthermore showed that *wbbZ*, encoded on the chromosome outside the O-locus, contributes to the diversity of the O-Ag structure displayed by *Kpn* and thus two isolates with the same O-locus can still result in different O-types based on the presence or absence of this gene. Immunologically relevant differences in O-Ag thus require the prediction of the O-Ag locus and *wbbZ* [29], so we added this to our O-Ag type predictions (Fig. S10A–D) where we thus distinguish between O1a and O1b as described by Kelly et al. [29]. Reflecting the trend observed with the ST types, we observe temporal variation in the prevalence of O-types and K-types (Fig. 5A and D, Fig. S10A). The non-ESBL population again displays a much greater diversity for both types of antigen (Fig. 5B and E, Fig. S10B), whilst the ESBL population is more biased towards dominant O- and K-types (Fig. 5C and F, Fig. S10C).

Beyond the dominant O1b (822/1373; 59.9%) and O2afg (184/1373; 13.4%) predicted O-antigen types, the infrequently observed type O4 comprises a substantial fraction of isolates (90/1373; 6.6%); and 35 isolates have a prediction accuracy as ‘Low’ or ‘None’ which can indicate either low assembly quality or novel O-Ag loci and types. In addition, we note two isolates with unknown O-Ag locus encoding for additional *wbbY* and *wbbZ* genes, indicating that more variation might be within possibly novel O-Ag loci depending on additional enzymes encoded in the chromosomal backbone (Table S5). We furthermore note 8 isolates with O1/O2 loci encoding for only *wbbZ* (not *wbbY*); whilst the recent work showed no structural differences in the O-Ag if *wbbZ* alone was added, we cannot exclude other carbohydrate enzymes on the chromosomes of our isolates that might lead to additional different structures if combined with *wbbZ* (Table S5). Of relevance, we also observe variation of O- and K-type combinations within the same sequence type (Fig. 5G and H, Fig. S10D) and with each other (Fig. 5I, Fig. S10E).

Considering hypothetical vaccine strategies and constructs, an efficacious maternally administered quadrivalent O-antigen-based vaccine (O1a, O1b, O2afg, O4;

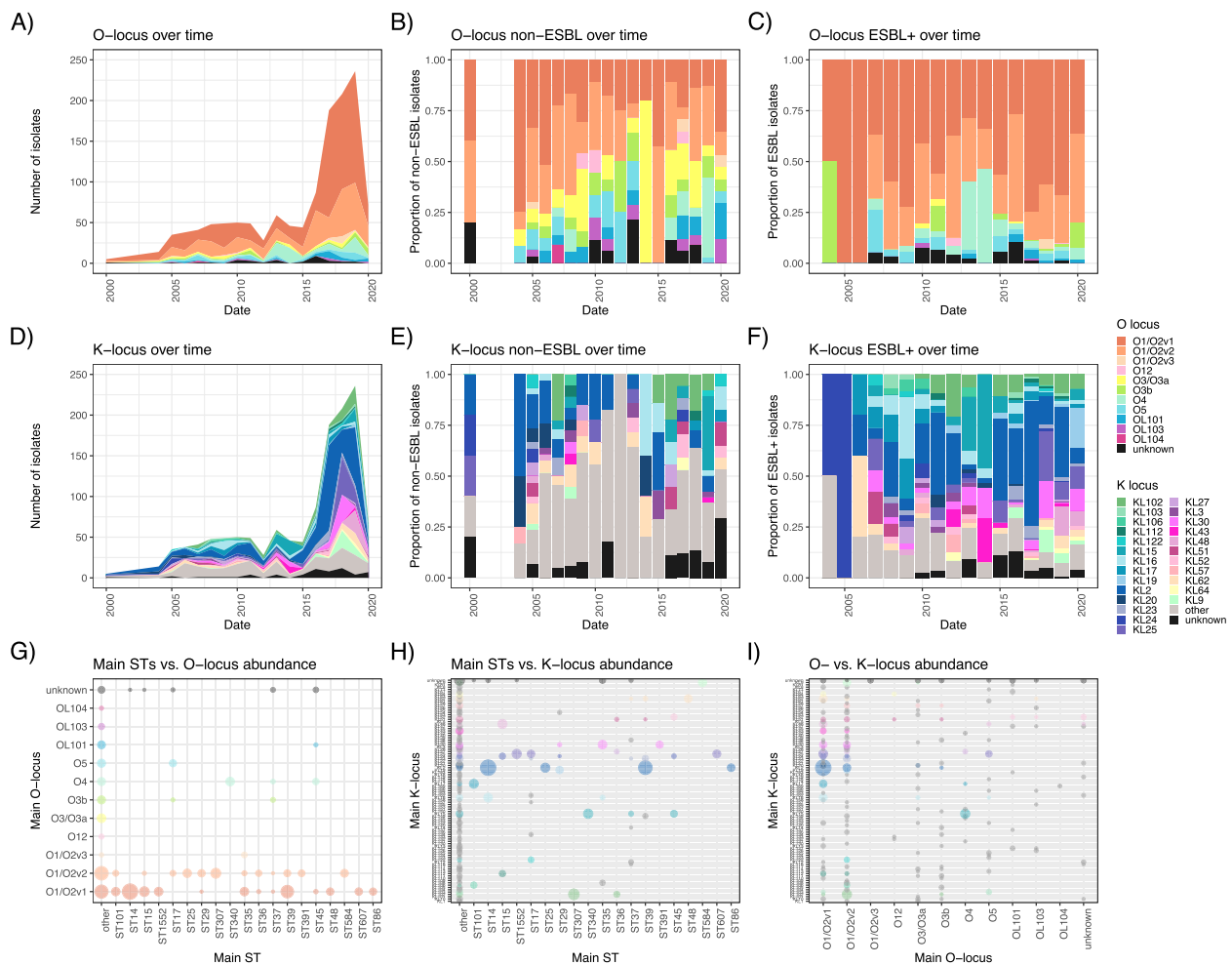


Fig. 5 High fluctuation of putative vaccine targets over time and diversity within major STs. The distribution of O-Ag locus types (top panels) and K-locus types (lower panels), resp., over time showing total numbers (**A** and **D**); and proportions of only isolates with ESBL resistance genes (**B** and **E**) or ESBL-negative isolates (**C** and **F**)

including O1a as O1b-encoding isolates can switch between O1a and O1b [29]) would be expected to protect against up to 89% of cases of neonatal sepsis in Blantyre (345/388), whereas a K-antigen based vaccine would require 22 K-antigens to be represented to protect against an equal number of cases of neonatal sepsis when taking the entire timespan of our study into account (KL10, KL102, KL103, KL104, KL106, KL108, KL117, KL142, KL15, KL16, KL17, KL19, KL2, KL23, KL25, KL30, KL43, KL48, KL51, KL52, KL62, KL9); a quadrivalent K-antigen based vaccine (KL2, KL25, KL30, KL102) would cover 58% (198/388) of cases. We furthermore observe no regular cycling between a specific set of K-types, but rather that different K-types regularly emerge associated with major clones (Fig. 5). Given current knowledge of > 180 predicted K-types [26] and > 70 determined structures so far [65–67] (likely an underestimation), and the propensity of the K-locus to recombine, it will be very

challenging to predict how long a K-type vaccine would remain effective.

Trends of resistance vs virulence determinants

Whilst most *Kpn* isolates are opportunistic pathogens with poorly understood virulence profiles, the hypermucoid phenotype is a known virulence determinant arising from hyperproduction of capsule and causing a different clinical infection type, i.e. liver abscess and meningitis in otherwise healthy individuals, often progressing very rapidly and with poor prognosis. The capsule hyperproduction is usually driven by acquisition of a virulence plasmid encoding for the capsule polysaccharide upregulation factor *rmpA2* as well as several siderophore systems (aerobactin, salmochelin, colistin) that enable iron scavenging. This phenotype is of particular concern given the recent convergence of hypervirulent with multidrug resistant isolates. Whilst we note hypervirulent isolates

present (defined here as *kleborate* virulence score > 1, i.e. encoding virulence factors in addition to the often chromosomal *yersiniabactin*) from the very early sampling times on in our collection (Fig. S11A,B, Fig. S5A, Fig. S6A, Fig. S7A), these remain sporadic; in contrast to multidrug-resistant isolates (Fig. S11C,D) are absent at these early sampling times but rapidly become the majority of isolates from 2007/8 onwards (Fig. S11D). Whilst we note a total number of > 10 isolates for both ST86 and ST268, two STs carrying the *rmpA2* gene and widely observed as hypervirulent phenotypes, these occur in different years and wards, highlighting that hospital-derived transmission is highly unlikely (Fig. S11B). Whilst it is of concern that we observe some of these potentially hypervirulent isolates (encoding for *rmpA2*) also encoding an ESBL gene (22 total; 9 of these ST86, no ST268) there seems to be no selective advantage; the first isolate carrying this combination is observed in 2010 but a maximum of 4 isolates per year are observed until 2020. We also noted one *K. variicola* isolate with predicted virulence genes; investigation using long-read sequencing confirms that the operon is present on the IncFIB/IncFII plasmid (Fig. S12). This strain also carries an IncFY plasmid, again highlighting that whilst represented in lower numbers, these species can readily acquire the same plasmids as *K. pneumoniae*. This small number of putative hypervirulent isolates is in accordance not only with bloodstream isolates in general but also with a study comparing carriage with infectious isolates from adults in Blantyre, where only a very small number of isolates encoding virulence determinants were identified, all of them in infectious isolates [68, 69].

Discussion

Neonatal sepsis is responsible for a high proportion of deaths in children under 5 in sSA, with *Klebsiella* by now being both the most prevalent gram-negative pathogen and increasingly hard to treat. We have analysed a longitudinal collection of routine diagnostics invasive (bloodstream and CSF) isolates of *Kpn* spanning from 1998 to 2020, during which time AMR increased drastically. We determined longitudinal patterns of patient demographics, *Kpn* population trends, vaccine targets, resistance elements and remaining treatment options. This exceptional resource, not only for sSA but also world-wide, enables us to understand the dynamics of *Kpn* over an exceptionally long time scale where the sampling was not biased towards resistance types. In particular, it allows us to see the dynamics before, during, and after the significant expansion of ESBL producing *Kpn* at maximum resolution; and highlights the need to consider not only major types based on total numbers but the temporal dynamics of vaccine targets such as cell surface polysaccharides.

Kpn initially showed a bimodal age distribution in patients at QECH [3] and there were concerns that increasing life expectancy would lead to a higher number of adult infections; however, this trend has yet to be seen, and adult bloodstream infections were significantly reduced overall following the widespread distribution of antiretroviral therapy [70]. The stark increase in infections from 2016 onwards is almost exclusively seen in neonates and children where ward-restricted clonal expansions that are subsequently replaced with other successful clones. This suggests that context appropriate, effective, scalable infection prevention and control (IPC) targeted towards the most vulnerable patient groups in the hospital and a better understanding of *Kpn* transmission routes and reservoirs would prevent a large proportion of the cases caused by *Klebsiella* bloodstream infection. Such approaches have the potential to protect vulnerable babies against all healthcare-associated infections rather than just one pathogen and are available now, unlike a vaccine strategy that will take years to develop and implement without guarantee for success.

The main sequence types in our study were ST39, ST14 and ST15. Whilst ST39 and ST14 are not necessarily the most prevalent ones observed in published data from other regions [5], ST15 is widely reported in SouthEast Asia [10] causing clonal outbreaks whereas it showed a different pattern in our setting driving smaller expansions but also as a lot of sporadic isolates occurring over time. This highlights the need for more data from sSA to understand the complex diversity of this organism in high-risk areas, as not only the ST composition, but also their patterns, differ between settings. The two key insights we gain from the population pattern is that the lineages continue to replace each other as the dominant clone over longer time periods, and that in our setting, this expansion is mainly driven by an increase in cases in neonatal patients. Most of the major sequence types are present at a steady, much lower proportion, before and after their respective clonal expansions, emphasising the relevance of understanding what leads to the wave-like pattern of clonal expansions in *Kpn* populations. Our previous work [68] on *Kpn* carriage and acquisitions in adults, in the hospital and in the community, showed similar STs present in community and hospital, but hospital infections to be biased towards a few STs. Introduction of the strains causing neonatal infections into the hospital could be through healthcare personnel, medical devices, parents or caretakers; and some of these STs then proceed to spread successfully in the hospital.

We have previously described the prolonged impact of ceftriaxone on selection for ESBL-Enterobacterales, including *Kpn* in adult patients in our setting, and there is a need to strengthen antimicrobial stewardship to reduce

unnecessary use of this agent alongside IPC interventions [68]. The temporal *Kpn* variability also favours prioritisation of IPC strategies to prevent the introduction of new lineages. It may be that increased IPC efforts in response to ward outbreaks contribute to or are the single cause for the sudden stops of clonal expansions. We have little understanding of the ability of *Kpn* to survive on abiotic surfaces, which represents one of the key differences between HIC and LMIC settings, as the latter often use wooden furniture and bristled plastic tubing due to limited resources, which might lead to different *Kpn* lineages being successful. Recent work on dry surface biofilms [71] has shown that the respective strain was able to survive over prolonged periods, further highlighting our need for a better understanding of biofilm formation and persistence on different hospital materials and how appropriate recommended IPC measures are for current clinical clones. A similar question remains open regarding agents used for disinfection, as resource-limited settings often rely on basic soap agents and use unsafe water, whilst HIC routinely use industrially produced, quality-controlled disinfectants.

Our data allow insight into AMR patterns in our setting. Ceftriaxone resistance, almost exclusively due to the ESBL-encoding *bla*_{CTX-M-15}, is widespread. Whilst carbapenems are rarely available and resistance is already emerging [72, 73], several treatment options are still viable, such as amikacin. Our analysis also emphasises again that aminoglycoside resistance is notoriously hard to predict and might be impacted by the genetic background (for example efflux pumps) and not only acquired resistance genes, and should therefore at this stage not be ruled out based on genomic data but confirmed via phenotyping. Most isolates were phenotypically resistant to gentamicin; whether this is consequent on the predicted resistance genes, or whether other mechanisms drive this resistance is important to determine to improve the value of genotypic predictions for aminoglycosides. It will remain to be further investigated whether this is due to other interfering genes, low expression of the resistance gene, or heteroresistance in the population; which needs to be carefully considered [74]. We furthermore observe a trend in re-emergence of chloramphenicol sensitivity, putatively in part driven by the spread of a non-functional CAT copy (CatB4) [57]. Continuing to monitor re-emergence of susceptibility to agents no longer in use is of high relevance to allow timely re-introduction of drugs and can give us insights into the potential of rotating antimicrobial regimens over longer time spans to allow sensitivity to re-emerge.

There was little resistance predicted and observed to colistin, a last-line treatment option, in QECH *Kpn* isolates. Especially the lack of mobile resistance elements is

encouraging, but also stresses the urgent need for better management of animal health as colistin is widely used, knowingly and as part of food products farmers are not necessarily aware of, in the increasing mid-size farming industry [75]. Another exciting new development are initial results of combining fosfomycin and flomoxef [76]; we observe only sporadic fosfomycin resistance mechanisms and very little spread of *ampC* genes, the only non-carbapenemases that would deactivate flomoxef, although we note that there are also counterindications for fosfomycin use in invasive infections.

As extensively resistant bacterial pathogens emerge and spread, there is an increasing urgency to develop vaccines to break the arms race between new antimicrobials and new resistance mechanisms. In this case, there is interest in developing maternally administered *Klebsiella* vaccines to prevent neonatal sepsis. A target of high interest is the extracellular polysaccharides that can be typed based on their respective operons, which are assigned different K-types (capsular antigen) and O-types (the lipopolysaccharide O-antigen chain). The K-antigen shows a very high diversity; and whilst we can identify several dominant types in our study, the long timeframe of our study highlights the importance of considering the dynamic nature of K-antigens when considering vaccine design. Here, temporal fluctuations detected just in Blantyre strongly suggest that a K-type vaccine would become ineffective very rapidly given the frequent changes in dominant K-type, which might be driven by phage pressure in the environment and thus highly unpredictable. It is also unclear if successful vaccination against the major polysaccharide types would lead to a degree of cross-protection against non-targeted types or whether lack of such cross-protection would facilitate rapid emergence of infections caused by vaccine escape lineages.

In contrast, there are far fewer O-antigen types, making this a more attractive target. Recent efforts have compared several large datasets with O- and K-typing either determined by PCR or WGS [77]; and identified that a conjugate vaccine targeting O1v1/2 (~serotype O1) [26], O2v2 (~serotype O2afg) [26] and O3b (~serotype O3b) [26] has the potential to prevent the majority (>70%) of cases of *Kpn*-BSI. For the most at-risk population in our setting, the neonates, this would cover 87% of cases (338/388) assuming O1a and O1b were included into O1 and no other subtypes were discovered [29]; importantly it would however not have prevented the ST340 outbreak in the neonatal unit previously described [19] (O4). It thus seems of relevance to consider including the currently low-abundance O-antigens as well, in particular given the interchangeability for O-types within the same STs. Reports from other highly understudied settings also report other main O-types in hospital isolates,

e.g. O5 in a study in Pakistan [52, 78] or a diversity of O-types in the Caribbean where this O-Ag vaccine would only cover 62% (162/260) [79]. Selection pressure might not directly drive evolution given that *Kpn* is not human restricted but could likely select for strains with currently less abundant O-types to increase in prevalence. Further, there remains an open question of how accessible the O-antigens are to antibodies, which a recent study calls into question [80]; we currently have very limited understanding what drives the extent of capsule expression per cell in non-hypermucoid isolates and how different stages of the infection impact this. It is known from in vitro studies that capsule expression even in the same experimental setting can differ widely within clonal lineages, which also impacts the bacterial ability to escape the complement system [81].

Our data, and the recent identification of a novel form of the long-studied O1 antigen type [29, 82–84], highlights that we have very little understanding of the relevance of the O- and K-types on the success of *Kpn* lineages, whether any additional genes on the chromosomal backbone can further modify the structures and lead to different serotypes with the same O-locus, as well as whether any O-/K-combinations are more or less favourable. Closing such knowledge gaps will be central to design of vaccines as we also have little understanding for the propensity of sequence type combinations with specific vaccine target serotypes such as capsule or O-antigen. A more solid understanding of O-/K-type variations, both regarding their biochemistry and whether they contribute to epidemiological success, is crucial given the high interest to use these as targets for maternally administered vaccines against neonatal sepsis.

This highlights that we have very little understanding of the relevance of the O- and K-types on the success of *Kpn* lineages, whether any additional genes on the chromosomal backbone can further modify the structures and lead to different serotypes with the same O-locus, as well as whether any O-/K-combinations are more or less favourable. A more solid understanding of O-/K-type variations, both regarding their biochemistry and whether they contribute to epidemiological success, is crucial given the high interest to use these as targets for maternally administered vaccines against neonatal sepsis.

Conclusions

In summary, our study provides a unique insight into long-term patterns of *Kpn* hospital isolates from a low-income setting, spanning across a time period where the number of MDR and ESBL-producing infections increased rapidly across the globe. We show that whilst STs known as high-risk in other settings are

common, these do not always show similar success in different settings; and we observe a significant number of isolates from otherwise rarely observed STs, causing hospital infections. This again emphasises the importance of including the relevant settings into studies aiming to identify treatment or vaccine targets as we currently have a poor understanding of what makes an ST a high-risk lineage in each setting at a given time. Our data also emphasises the urgent need to consider temporal variation when considering treatment or vaccine targets, as some of the main vaccine targets show strong temporal fluctuations; the most prevalent type now circulating is unlikely to cause the next outbreak. The close relatedness of isolates from the same ward in the same time frame strongly indicates that the majority of infections are hospital-acquired, so whilst vaccine development and alternative treatment options are highly relevant; at this stage, focused efforts to improve IPC are key to reducing *Kpn* disease burden now [56].

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13073-024-01342-3>.

Additional file 1: Supplementary figures Fig.S1-Fig.S12 and respective figure legends and a description of Tables S1-S6.

Additional file 2: Supplementary tables Table S1-Table S6 as worksheets in one xls file.

Acknowledgements

We would like to thank the Sanger Institute Pathogen Genomics group for expert computational support. We acknowledge and thank all the clinical staff and patients at QECH over the years for their contribution; and the MLW core microbiology team for expert support in storage and handling of the isolates.

Authors' contributions

Conceptualization: EH, NAF; methodology: EH, NAF; software: EH, JML; validation: EH, OP, AZ; formal analysis: EH, OP; investigation: EH, OP, AZ, PM, PS, ET, FG, RL, SL, JC, JML; resources: SB, PS, ET, KK, NRT, NAF; data curation: EH, OP, PM, RL, JC, JML, AZ; writing—original draft: EH, OP, NAF; writing—review and editing: all authors; visualisation: EH, OP, JML, NAF; supervision: EH, CM, SL, KK, NAF; project administration: EH, NAF; funding acquisition: EH, NRT, NAF. All authors read and approved the final manuscript.

Funding

We acknowledge funding from Wellcome (EH; grant 217303/Z/19/Z) and the BMGF (NAF and EH; grant INV-005180) as well as Wellcome funding providing core support for the Wellcome Sanger Institute (206194) and MLW (206454). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

All sequence data is available on SRA/ENA under BioProject PRJEB42462 <https://www.ebi.ac.uk/ena/browser/view/PRJEB42462> (short read data) and PRJNA1102521 <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1102521> (long-read data); specific accessions and all additional data is provided in the supporting tables S1-S6. All data and code used to create the analyses and is available as R package QECHospitalKlebs v0.0.9 (<https://github.com/EvitaH/QECHospitalKlebs>; <https://doi.org/10.5281/zenodo.8421658>).

Declarations

Ethics approval and consent to participate

The *Klebsiella* Isolates sequenced for this study were originally generated by the routine clinical diagnostic microbiology service at QECH, therefore individual level consent was not required. Ethical approval for this study was granted by the University of Malawi College of Medicine Research Ethics Committee (COMREC) (P.11/18/2541) and isolates were shipped under a Nagoya Protocol compliant Access and Benefit Sharing agreement between the Government of Malawi and the Wellcome Sanger Institute. The research conformed to the principle of the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Vector Biology, Liverpool School of Tropical Medicine, Pembroke Place Liverpool L3 5QA, UK. ²Department of Clinical Sciences, Liverpool School of Tropical Medicine, Pembroke Place Liverpool L3 5QA, UK. ³Malawi Liverpool Wellcome Programme, Kamuzu University of Health Sciences, Blantyre, Malawi. ⁴Kamuzu University of Health Sciences, Blantyre, Malawi. ⁵Queen Elizabeth Central Hospital, Blantyre, Malawi. ⁶Division of Infection & Immunity, University College London, London, UK. ⁷Department of Clinical Infection, Microbiology and Immunology, University of Liverpool, Liverpool, UK. ⁸Parasites and Microbes Program, Wellcome Sanger Institute, Hinxton, UK. ⁹London School of Hygiene and Tropical Medicine, London, UK. ¹⁰School of Medicine, St Andrews University, St Andrews, UK.

Received: 10 October 2023 Accepted: 30 April 2024

Published online: 06 May 2024

References

- Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet Lond Engl*. 2016;388(10063):3027–35.
- Sands K, Carvalho MJ, Portal E, Thomson K, Dyer C, Akpulu C, et al. Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. *Nat Microbiol*. 2016;6(4):512–23.
- Musicha P, Cornick JE, Bar-Zeev N, French N, Masera C, Denis B, 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(10):1042–52.
- Salzberg NT, Sivalogan K, Bassat Q, Taylor AW, Adedini S, El Arifeen S, et al. Mortality surveillance methods to identify and characterize deaths in child health and mortality prevention surveillance network sites. *Clin Infect Dis*. 2019;69(Supplement_4):S262–73.
- Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. *Nat Rev Microbiol*. 2020;18:344–59.
- Ellington MJ, Heinz E, Wailan AM, Dorman MJ, de Goffau M, Cain AK, et al. Contrasting patterns of longitudinal population dynamics and antimicrobial resistance mechanisms in two priority bacterial pathogens over 7 years in a single center. *Genome Biol*. 2019;20(1):184.
- Lipworth S, Vihta KD, Chau K, Barker L, George S, Kavanagh J, et al. Ten-year longitudinal molecular epidemiology study of *Escherichia coli* and *Klebsiella* species bloodstream infections in Oxfordshire, UK. *Genome Med*. 2021;13(1):144.
- Fostervold A, Hetland MAK, Bakksjø R, Bernhoff E, Holt KE, Samuelson Ø, et al. A nationwide genomic study of clinical *Klebsiella pneumoniae* in Norway 2001–15: introduction and spread of ESBLs facilitated by clonal groups CG15 and CG307. *J Antimicrob Chemother*. 2022;77(3):665–74.
- Thorpe H, Booton R, Kallonen T, Gibbon MJ, Couto N, Passet V, et al. One Health or Three? Transmission modelling of *Klebsiella* isolates reveals ecological barriers to transmission between humans, animals and the environment. *Microbiology*. 2021. <https://doi.org/10.1102/2021.08.05.455249>.
- Wyres KL, Nguyen TNT, Lam MMC, Judd LM, van Vinh CN, Dance DAB, et al. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiellapneumoniae* from South and Southeast Asia. *Genome Med*. 2020;12(1):11.
- Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol*. 2014;15(3):R46.
- Bankevic A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol J Comput Mol Cell Biol*. 2012;19(5):455–77.
- Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, Harris SR, et al. Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data. *Microb Genomics*. 2016;2(8):e000083.
- Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinforma Oxf Engl*. 2014;30(14):2068–9.
- Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol*. 2019;37(5):540–6.
- Wick RR, Holt KE. Polypolish: Short-read polishing of long-read bacterial genome assemblies. Schneidman-Duhovny D, editor. *PLoS Comput Biol*. 2022;18(1):e1009802.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother*. 2014;58(7):3895–903.
- Lester R, Musicha P, Kawaza K, Langton J, Mango J, Mangochi H, et al. Effect of resistance to third-generation cephalosporins on morbidity and mortality from bloodstream infections in Blantyre, Malawi: a prospective cohort study. *Lancet Microbe*. 2022;3(12):e922–30.
- Cornick J, Musicha P, Peno C, Seager E, Iroh Tam PY, Bilima S, et al. Genomic investigation of a suspected *Klebsiellapneumoniae* outbreak in a neonatal care unit in sub-Saharan Africa. *Microb Genomics*. 2021;7(11):000703.
- Musicha P, Msefula CL, Mather AE, Chaguzo C, Cain AK, Peno C, et al. Genomic analysis of *Klebsiellapneumoniae* isolates from Malawi reveals acquisition of multiple ESBL determinants across diverse lineages. *J Antimicrob Chemother*. 2019;74(5):1223–32.
- Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microb Genomics*. 2017;3(10):e000131.
- Inouye M, Dashnow H, Raven LA, Schultz MB, Pope BJ, Tomita T, et al. SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med*. 2014;6(1):90.
- Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob Agents Chemother*. 2014;58(1):212–20.
- McArthur AG, Waghechner N, Nizam F, Yan A, Azad MA, Baylay AJ, et al. The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother*. 2013;57(7):3348–57.
- Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for *Klebsiellapneumoniae* and its related species complex. *Nat Commun*. 2021;12(1):4188.
- Lam MMC, Wick RR, Judd LM, Holt KE, Wyres KL. Kaptive 2.0: updated capsule and lipopolysaccharide locus typing for the *Klebsiellapneumoniae* species complex. *Microb Genomics*. 2022;8:000800.
- Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* Genomes. Diekema DJ, editor. *J Clin Microbiol*. 2018;56(6):e00197–18 /jcm/56/6/e00197-18.atom.
- Follador R, Heinz E, Wyres KL, Ellington MJ, Kowarik M, Holt KE, et al. The diversity of *Klebsiellapneumoniae* surface polysaccharides. *Microb Genomics*. 2016;2(8):e000073.
- Kelly SD, Ovchinnikova OG, Müller F, Steffen M, Braun M, Sweeney RP, et al. Identification of a second glycoform of the clinically prevalent O1 antigen from *Klebsiellapneumoniae*. *Proc Natl Acad Sci U S A*. 2023;120(29):e2301302120.
- Torsten Seemann. snippy: fast bacterial variant calling from NGS reads. Available from: <https://github.com/tseemann/snippy>

31. Wu KM, Li LH, Yan JJ, Tsao N, Liao TL, Tsai HC, et al. Genome sequencing and comparative analysis of *Klebsiella pneumoniae* NTUH-K2044, a strain causing liver abscess and meningitis. *J Bacteriol*. 2009;191(14):4492–501.
32. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res*. 2015;43(3):e15–e15.
33. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol Biol Evol*. 2009;26(7):1641–50.
34. Elliott AG, Ganesamoorthy D, Coin L, Cooper MA, Cao MD. Complete genome sequence of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* Strain ATCC 700603. *Genome Announc*. 2016;4(3):e00438–16.
35. Pinto-Tomás AA, Anderson MA, Suen G, Stevenson DM, Chu FST, Cleland WW, et al. Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. *Science*. 2009;326(5956):1120–3.
36. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genomics*. 2016;2(4):e000056.
37. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*. 2015;32(1):268–74.
38. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods*. 2017;14(6):587–9.
39. Pupko T, Pe'er I, Shamir R, Graur D. A fast algorithm for joint reconstruction of ancestral amino acid sequences. *Mol Biol Evol*. 2000;17(6):890–6.
40. Waillan AM, Coll F, Heinz E, Tonkin-Hill G, Corander J, Feasey NA, et al. rPinecone: define sub-lineages of a clonal expansion via a phylogenetic tree. *Microb Genomics*. 2019;5(4):e000264.
41. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res*. 2021;49(W1):W293–6.
42. Shen Z, Gao Q, Qin J, Liu Y, Li M. Emergence of an NDM-5-Producing Hypervirulent *Klebsiella pneumoniae* Sequence Type 35 Strain with Chromosomal Integration of an Integrative and Conjugative Element, ICE *Kp1*. *Antimicrob Agents Chemother*. 2019;64(1):e01675–e1719.
43. Cabanel N, Rosinski-Chupin I, Chiarelli A, Botin T, Tato M, Canton R, et al. Evolution of VIM-1-producing *Klebsiella pneumoniae* isolates from a hospital outbreak reveals the genetic bases of the loss of the urease-positive identification character. Beiko RG, editor. *MSystems*. 2021;6(3):e00244–21.
44. Stoesser N, Giess A, Batty EM, Sheppard AE, Walker AS, Wilson DJ, et al. Genome sequencing of an extended series of NDM-producing *Klebsiella pneumoniae* isolates from neonatal infections in a Nepali hospital characterizes the extent of community- versus hospital-associated transmission in an endemic setting. *Antimicrob Agents Chemother*. 2014;58(12):7347–57.
45. Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, et al. Producing polished prokaryotic pangenomes with the Panaroo pipeline. *Genome Biol*. 2020;21(1):180.
46. Wickham H. *Ggplot2: elegant graphics for data analysis*. New York: Springer; 2009. p. 212 (Use R!).
47. Yu G, Lam TTY, Zhu H, Guan Y. Two methods for mapping and visualizing associated data on phylogeny using ggtree. *Mol Biol Evol*. 2018;35(12):3041–3.
48. R Core Team. *A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna; 2021. Available from: <https://www.R-project.org>
49. World Health Organization, editor. *Antimicrobial resistance: global report on surveillance*. Geneva, Switzerland: World Health Organization; 2014. p. 232.
50. Rodrigues C, Passet V, Rakotondrasoa A, Diallo TA, Criscuolo A, Brisse S. Description of *Klebsiella laafricanensis* sp. nov., *Klebsiella variicola* subsp. *tropicalensis* subsp. nov. and *Klebsiella variicola* subsp. *variicola* subsp. nov. *Res Microbiol*. 2019;170(3):165–70.
51. Gordon MA, Walsh AL, Chaponda M, Soko D, Mbwinji M, Molyneux ME, et al. Bacteraemia and mortality among adult medical admissions in Malawi—predominance of non-typhi salmonellae and *Streptococcus pneumoniae*. *J Infect*. 2001;42(1):44–9.
52. Ejaz H, Wang N, Wilksch JJ, Page AJ, Cao H, Gujarani S, et al. Phylogenetic analysis of *Klebsiella pneumoniae* from hospitalized children. *Pakistan Emerg Infect Dis*. 2017;23(11):1872–5.
53. Chung The H, Karkey A, Pham Thanh D, Boinett CJ, Cain AK, Ellington M, et al. A high-resolution genomic analysis of multidrug-resistant hospital outbreaks of *Klebsiella pneumoniae*. *EMBO Mol Med*. 2015;7(3):227–39.
54. Wyres KL, Hawkey J, Hetland MAK, Fostervold A, Wick RR, Judd LM, et al. Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. *J Antimicrob Chemother*. 2019;74(3):577–81.
55. Gorrie CL, Da Silva AG, Ingle DJ, Higgs C, Seemann T, Stinear TP, et al. Key parameters for genomics-based real-time detection and tracking of multidrug-resistant bacteria: a systematic analysis. *Lancet Microbe*. 2021;2(11):e575–83.
56. Mangochi H, Tolhurst R, Simpson V, Kawaza K, Chidziwisano K, Feasey NA, et al. A qualitative study exploring hand hygiene practices in a neonatal unit in Blantyre, Malawi: implications for controlling healthcare-associated infections. *Wellcome Open Res*. 2022;7:146.
57. Graf FE, Goodman RN, Galichan S, Forrest S, Picton-Barlow E, Fraser AJ, et al. Molecular mechanisms of re-emerging chloramphenicol susceptibility in extended-spectrum beta-lactamase producing Enterobacteriales. *Microbiology*; 2023. <http://biorxiv.org/lookup/doi/10.1101/2023.11.16.567242>.
58. Sojo-Dorado J, López-Hernández I, Rosso-Fernandez C, Morales IM, Palacios-Baena ZR, Hernández-Torres A, et al. Effectiveness of fosfomycin for the treatment of multidrug-resistant *Escherichia coli* bacteremic urinary tract infections: a randomized clinical trial. *JAMA Netw Open*. 2022;5(1):e2137277.
59. McGovern PC, Wible M, El-Tahtawy A, Biswas P, Meyer RD. All-cause mortality imbalance in the tigecycline phase 3 and 4 clinical trials. *Int J Antimicrob Agents*. 2013;41(5):463–7.
60. Veleba M, Schneiders T. Tigecycline resistance can occur independently of the *ramA* Gene in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*. 2012;56(8):4466–7.
61. Zhang R, Dong N, Huang Y, Zhou H, Xie M, Chan EWC, et al. Evolution of tigecycline- and colistin-resistant CRKP (carbapenem-resistant *Klebsiella pneumoniae*) in vivo and its persistence in the GI tract. *Emerg Microbes Infect*. 2018;7(1):127.
62. Roy S, Datta S, Viswanathan R, Singh AK, Basu S. Tigecycline susceptibility in *Klebsiella pneumoniae* and *Escherichia coli* causing neonatal septicemia (2007–10) and role of an efflux pump in tigecycline non-susceptibility. *J Antimicrob Chemother*. 2013;68(5):1036–42.
63. He T, Wang R, Liu D, Walsh TR, Zhang R, Lv Y, et al. Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. *Nat Microbiol*. 2019;4(9):1450–6.
64. Huang Y, Rana AP, Wenzler E, Ozer EA, Krapp F, Bulitta JB, et al. Aminoglycoside-resistance gene signatures are predictive of aminoglycoside MICs for carbapenem-resistant *Klebsiella pneumoniae*. *J Antimicrob Chemother*. 2022;77(2):356–63.
65. Orskov I, Fife-Asbury MA. New *Klebsiella* capsular antigen, K82, and the deletion of five of those previously assigned. *Int J Syst Bacteriol*. 1977;27(4):386–7.
66. Edmunds PN. Further *Klebsiella* capsule types. *J Infect Dis*. 1954;94(1):65–71.
67. Edwards PR, Fife MA. Capsule types of *Klebsiella*. *J Infect Dis*. 1952;91(1):92–104.
68. Lewis JM, Mphasa M, Banda R, Beale MA, Heinz E, Mallewa J, et al. Colonization dynamics of extended-spectrum beta-lactamase-producing Enterobacteriales in the gut of Malawian adults. *Nat Microbiol*. 2022;7(10):1593–604.
69. Lewis JM, Mphasa M, Banda R, Beale MA, Mallewa J, Heinz E, et al. Genomic and antigenic diversity of colonizing *Klebsiella pneumoniae* isolates mirrors that of invasive isolates in Blantyre, Malawi. *Microb Genomics*. 2022;8(3):000778.
70. Feasey NA, Houston A, Mukaka M, Komrower D, Mwalukomo T, Tenthani L, et al. A reduction in adult blood stream infection and case fatality at a large african hospital following antiretroviral therapy roll-out. Pett S, editor. *PLoS One*. 2014;9(3):e92226.
71. Centeleghe I, Norville P, Hughes L, Maillard JY. *Klebsiella pneumoniae* survives on surfaces as a dry biofilm. *Am J Infect Control*. 2023;51(10):1157–62.

72. Lewis JM, Lester R, Mphasa M, Banda R, Edwards T, Thomson NR, et al. Emergence of carbapenemase-producing Enterobacteriaceae in Malawi. *J Glob Antimicrob Resist*. 2020;20:225–7.
73. Kumwenda GP, Sugawara Y, Abe R, Akeda Y, Kasambara W, Chizani K, et al. First Identification and genomic characterization of multidrug-resistant carbapenemase-producing Enterobacteriaceae clinical isolates in Malawi. *Africa J Med Microbiol*. 2019;68(12):1707–15.
74. Zhang F, Li Q, Bai J, Ding M, Yan X, Wang G, et al. Heteroresistance to Amikacin in Carbapenem-Resistant *Klebsiella pneumoniae* Strains. *Front Microbiol*. 2021;12:682239.
75. Mankhomwa J, Tolhurst R, M'biya E, Chikowe I, Banda P, Mussa J, et al. A qualitative study of antibiotic use practices in intensive small-scale farming in urban and peri-urban blantyre, malawi: implications for antimicrobial resistance. *Front Vet Sci*. 2022;9:876513.
76. Darlow CA, Farrington N, Johnson A, McEntee L, Unsworth J, Jimenez-Valverde A, et al. Flomoxef and fosfomycin in combination for the treatment of neonatal sepsis in the setting of highly prevalent antimicrobial resistance. *J Antimicrob Chemother*. 2022;77(5):1334–43.
77. Lipworth S, Vihta KD, Chau KK, Kavanagh J, Davies T, George S, et al. Ten years of population-level genomic *Escherichiacoli* and *Klebsiellapneumoniae* serotype surveillance informs vaccine development for invasive infections. *Clin Infect Dis*. 2021;73(12):2276–82.
78. Heinz E, Ejaz H, Bartholdson Scott J, Wang N, Gujran S, Pickard D, et al. Resistance mechanisms and population structure of highly drug resistant *Klebsiella* in Pakistan during the introduction of the carbapenemase NDM-1. *Sci Rep*. 2019;9(1):2392.
79. Heinz E, Brindle R, Morgan-McCalla A, Peters K, Thomson NR. Caribbean multi-centre study of *Klebsiellapneumoniae*: whole-genome sequencing, antimicrobial resistance and virulence factors. *Microb Genomics*. 2019;5(5):e000266.
80. Wantuch PL, Koot CJ, Robinson LS, Vinogradov E, Scott NE, Harding CM, et al. Capsular polysaccharide inhibits vaccine-induced O-antigen antibody binding and function across both classical and hypervirulent K2:O1 strains of *Klebsiellapneumoniae*. *Hakansson AP, editor. PLoS Pathog*. 2023;19(5):e1011367.
81. Loraine J, Heinz E, De Sousa Almeida J, Milevskyy O, Voravuthikunchai SP, Srimanote P, et al. Complement susceptibility in relation to genome sequence of recent *Klebsiellapneumoniae* isolates from thai hospitals. *mSphere*. 2018;3(6):e00537–18.
82. Kol O, Wieruszkeski JM, Strecker G, Fournet B, Zalisz R, Smets P. Structure of the O-specific polysaccharide chain of *Klebsiella pneumoniae* O1K2 (NCTC 5055) lipopolysaccharide. A complementary elucidation. *Carbohydr Res*. 1992;15(236):339–44.
83. Whitfield C, Richards JC, Perry MB, Clarke BR, MacLean LL. Expression of two structurally distinct D-galactan O antigens in the lipopolysaccharide of *Klebsiella pneumoniae* serotype O1. *J Bacteriol*. 1991;173(4):1420–31.
84. Kol O, Wieruszkeski JM, Strecker G, Montreuil J, Fournet B, Zalisz R, et al. Structure of the O-specific polysaccharide chain from *Klebsiella pneumoniae* O1K2 (NCTC 5055) lipopolysaccharide. *Carbohydr Res*. 1991;18(217):117–25.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.