MICROBIAL GENOMICS

RESEARCH ARTICLE
Perlaza-Jiménez *et al.*, *Microbial Genomics* 2020;6
DOI 10.1099/mgen.0.000433

MICROBIOLOGY SOCIETY
OPEN DATA OPEN ACCESS

Forensic genomics of a novel *Klebsiella quasipneumoniae* type from a neonatal intensive care unit in China reveals patterns of colonization, evolution and epidemiology

Laura Perlaza-Jiménez[1], Qing Wu[1], Von Vergel L. Torres[2], Xiaoxiao Zhang[3], Jiahui Li[1,2], Andrea Rocker[2], Trevor Lithgow[2,*], Tieli Zhou[1,*] and Dhanasekaran Vijaykrishna[1,2,*]

Abstract

During March 2017, a neonatal patient with severe diarrhoea subsequently developed septicaemia and died, with *Klebsiella* isolated as the causative microorganism. In keeping with infection control protocols, the coincident illness of an attending staff member and three other neonates with *Klebsiella* infection triggered an outbreak response, leading to microbiological assessment of isolates collected from the staff member and all 21 co-housed neonates. Multilocus sequence typing and genomic sequencing identified that the isolates from the 21 neonates were of a new *Klebsiella* sequence type, ST2727, and taxonomically belonged to *K. quasipneumoniae* subsp. *similipneumoniae* (formerly referred to as KpIIB). Genomic characterization showed that the isolated ST2727 strains had diverged from other *K. quasipneumoniae* subsp. *similipneumoniae* strains at least 90 years ago, whereas the neonatal samples were highly similar with a genomic divergence of 3.6 months. There was no relationship to the *Klebsiella* isolate from the staff member. This demonstrates that no transmission occurred from staff to patient or between patients. Rather, the data suggest that ST2727 colonized each neonate from a common hospital source. Sequence-based analysis of the genomes revealed several genes for antimicrobial resistance and some virulence features, but suggest that ST2727 is neither extremely-drug resistant nor hypervirulent. Our results highlight the clinical significance and genomic properties of ST2727 and urge genome-based measures be implemented for diagnostics and surveillance within hospital environments. Additionally, the present study demonstrates the need to scale the power of genomic analysis in retrospective studies where relatively few samples are available.

DATA SUMMARY

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession numbers JAAOBC000000000 to JAAOBX000000000, BioProject ID PRJNA610124. All bioinformatic protocols used to process the genomic data are available at https://github.com/vjlab/KpIIB_ST2727.

INTRODUCTION

Species of *Klebsiella* are widespread in the environment, found in soil and ground-water, as commensal organisms on plants and are carried widely in animal hosts [1]. *Klebsiella pneumoniae* is recognized as a leading cause of healthcare-associated urinary tract infections, surgical site infections and pneumonia, increasingly threatening neonates and the immunocompromised [2]. However, in recent years, an increasing diversity of *Klebsiella* species have been identified to cause infection in humans [3, 4]. Genome sequencing has revealed that bacteria historically classified as *K. pneumoniae* belonged to the *K. pneumoniae* species complex containing multiple phylogenetically distinct but closely related bacterial species. These include *K. pneumoniae* (previously classified as phylogroup KpI), *K. quasipneumoniae* subsp.

Received 17 March 2020; Accepted 01 September 2020; Published 15 September 2020
Author affiliations: [1]The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, PR China; [2]Infection and Immunity Program, Biomedicine Discovery Institute and Department of Microbiology, Monash University, Australia; [3]Women's Hospital School of Medicine Zhejiang University, Hangzhou, PR China.
*Correspondence: Trevor Lithgow, trevor.lithgow@monash.edu; Tieli Zhou, wyztli@163.com; Dhanasekaran Vijaykrishna, vijay.dhanasekaran@monash.edu
Keywords: *Klebsiella quasipneumoniae* subsp. *similipneumoniae*; genetic diversity; virulence determinants; antimicrobial resistance.
Abbreviations: AMR, antimicrobial resistance; hv, hyper-virulent; MIC, minimum inhibitory concentration; MKT, McDonald–Kreitman Test; ML, maximum-likelihood; MLST, multi-locus sequence typing; NI, neutrality index; NICU, neonatal intensive care unit; SNV, single nucleotide variation; ST, sequence type; TMRCA, time to the most recent common ancestor; T3SS, type 3 secretion system; WGS, whole genome sequencing.
Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Seven supplementary tables and two supplementary figures are available with the online version of this article.
000433 © 2020 The Authors

This is an open-access article distributed under the terms of the Creative Commons Attribution NonCommercial License. This article was made open access via a Publish and Read agreement between the Microbiology Society and the corresponding author's institution.

quasipneumoniae (previously KpIIA) and subsp. *similipneumoniae* (previously KPIIB), *K. variicola* (previously KPIII) [5], as well as two strains forming divergent lineages indicating potentially novel species within this species complex [6]. Whole genome sequencing (WGS) has shown that the predominant cause of clinical infection worldwide has been *K. pneumoniae* (KpI) [5] but there is an increasing trend, and increasing concern, in the detection of severe cases due to the newly described species [7]. Different studies report *K. quasipneumoniae* isolates causing infections resistant to treatments with ceftazidime and other oxyimino-betalactam antibiotics, and widespread carriage of genes encoding carbapenemase (KPC) [3, 4, 8–11].

With the emergence of a greater diversity of *Klebsiella* causing severe infections, their ability to colonize environmental niches such as sinks and ventilators in hospitals [12], increased detection of hybrid strains with drug-resistant and hypervirulent (hv) phenotypes, especially in regions such as China where the earliest reports of hvKp originated about three decades ago [13], an early warning system for the detection of *Klebsiella* species is being promoted in hospitals in China [14]. Routine surveillance is conducted using multilocus sequence typing (MLST) and followed by antimicrobial and virulence testing to provide an avenue to investigate potential outbreaks and develop better health control measures.

Here we report a retrospective study of an incident that occurred in March 2017, in the neonatal intensive care unit (NICU) of a major hospital in China with >3000 beds. A neonate with severe diarrhoea subsequently developed septicaemia and died, with *Klebsiella* isolated as the causative microorganism from a blood sample. During the same period, severe symptoms of diarrhoea were found in three other neonatal patients as well as an attending NICU staff member, triggering a response to quell the apparent outbreak. In addition to intensified hygiene procedures, the response included MLST and microbiological antimicrobial resistance (AMR) screening of stool samples from patients (21 neonates) and the staff member. Preliminary microbiological characterization of the samples revealed that 20 of the isolates taken from the infants belonged to a new MLST designation, ST2727, and one infant was infected with ST477. The sample from the staff member was of ST23 type, a well-defined hypervirulent clonal group frequently detected in severe cases of infection, whereas little was known of ST2727 or ST477. To forensically dissect the details of this case, we generated whole genome data for all bacterial isolates and carried out a detailed genomic characterization of virulence and AMR. Using a globally sampled dataset comprising 3611 genomes we elucidate the overall genetic diversity, evolution and epidemiology to illuminate elements of nosocomial *Klebsiella* transmission and ecological colonization that impact generally on understanding hospital-acquired infections.

Impact Statement

Colonization of the neonatal gut is a complex process with contributions from the mother's microbiome and environmental sources. In this study, 21 neonates housed in a single room were sampled across 22 days for the carriage of *Klebsiella* in response to an outbreak-status event. To forensically dissect the details of this case, we generated whole genome data for all bacterial isolates and carried out a detailed genomic characterization of virulence and antimicrobial resistance. A major outcome from this retrospective, deep, sequence analysis is the detection of colonization of the neonates by an emergent multilocus sequence type, ST2727, strain of *K. quasipneumoniae* subsp. *similipneumoniae* from a neonatal intensive care unit (NICU) environment, underscoring the need for both genomic surveillance and preventative measures in NICU environments.

METHODS

Collection of clinical isolates and clinical data

Klebsiella was isolated from the blood sample taken prior to death of the neonate, and the stool samples collected from all 21 infants in the NICU and a staff member who suffered an episode of enteritis. These isolates were characterized using a Vitek II system (bioMérieux). Relevant clinical characteristics of the patients and staff were taken from their medical records, including demographic characteristics, underlying medical conditions, clinical manifestations, treatments and outcomes. The study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).

Molecular genotyping and sequence typing of isolates

Bacterial isolates were characterized by PFGE after digestion of genomic DNA samples with *Xba*I under the following conditions: temperature 14 °C, voltage 6 V cm^{-1}, pulse angle 120°, and pulse duration of 5–35 s for 18 h, using a well-characterized *Xba*I digest (Takara Bio) of *Salmonella enterica* serotype H9812 as a molecular marker. DNA digest patterns were analysed and interpreted according to Tenover *et al.* [15]. MLST was carried out by PCR-amplifying seven housekeeping genes: *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB* [16], and the data were used to assign sequence types (STs) using the Institute Pasteur MLST database (https://bigsdb.pasteur.fr/). Through this process, the new ST2727 was established. Additionally, a standard multiplex-PCR was used to confirm that the isolates did not contain any of the five virulent extracellular polysaccharide capsules (CPS) (K antigen status; K-type) K1, K2, K20, K54 and K57.

Bacterial genome sequencing and assembly

DNA was extracted using a Biospin Bacterial Genomic DNA extraction kit (Hangzhou Bioer Technology). Libraries were prepared using the TruePrepTM DNA Library Prep Kit V2 for Illumina (Vazyme). Briefly, the DNA sample was fragmented and tagged with adapters using a single transposase enzymatic reaction, followed by amplification using an optimized, limited-cycle PCR protocol and indexing. Individual libraries were assessed on the QIAxcel Advanced Automatic nucleic acid

analyser, and quantified through quantitative PCR using a KAPA SYBR FAST qPCR Kit. Illumina Next Generation Sequencing technology (Hiseq PE150) was used to generate a paired-end library of short read sequences (150 nt) for each isolate. Sequence quality controls were performed using FastQC version 10-01-18 and trimmed and refined using Fastx toolkit version 0.0.13. *De novo* assembly was performed using SPAdes version 3.13.1 [17]. Scaffold generation and ordering were performed using the MeDuSa version available in July 2018 and Mauve version 2.4.0, respectively [18, 19]. Closely related genomes [NTUH-K2044 (Kp9), CP027602 (Kp10), HKUOPLC (Kp1–8, Kp11–22)] from NCBI were identified based on genome identity using MagicBlast v1.5 [20]. Upon assembly and alignment of all ST2727 genomes, single nucleotide variations (SNVs) were inferred using the Parsnp tool from Harvest Suite version 1.1.2 using a reference Kp1 isolate genome [NCBI accession numbers JAAOBC000000000 to JAAOBX000000000; Table S1 (available in the online version of this article)] [21].

Genomic analysis

Genes contributing to drug resistance and virulence were detected using Kaptive version available in July 2018 and Kleborate version 0.3.0 [22, 23]. VRprofile version 2.0 was used to detect gene clusters characteristic of protein secretion systems of type 3 (T3SS), type 4 (T4SS) or type 6 (T6SS) [24]. The presence of genes encoding a type 2 secretion system (T2SS) was detected by BLAST version 2.7.1, using as queries known T2SS genes [25]. Phylogenetic analysis was conducted using 3611 *Klebsiella* genomes downloaded from the NCBI database (downloaded on 20 June 2018) to find the closest related genomes to the novel strains (Data S1). Core genome alignment of *Klebsiella* genomes (including ST2727 isolates) was generated using Roary version 3.11.2. Excluding poorly assembled genomes, the final dataset constituted 3611 genomes comprising 3251 genes that were in at least 99% of the sequences [26]. Phylogenetic relationships were estimated using the maximum-likelihood (ML) method in RAxML version 8.2.12 [27] using the General Time Reversible (GTR) nucleotide substitution model with a gamma (Γ) distribution of among-site rates. Branch support was estimated using an ML bootstrap analysis with from 10 to >1000 replicates for the different datasets analysed. Additionally, the SNVs found in the core genome of all ST2727 isolates were concatenated to reconstruct a phylogenetic tree using the program SplitsTree v.4. and a BioNJ algorithm [28].

K. quasipneumoniae subsp. *similipneumoniae* (KpIIB) genomes were used to determine the evolutionary rate of this clade. To confirm that the genome datasets contained sufficient genetic change between sampling times, necessary for the reconstruction of the timescale of evolution, we used a root-to-tip regression of sampling years against genetic diversity in TempEst v1.5 [29], optimizing the best fit for the root to maximize the determination coefficient, R^2. The slope of the regression was positive, showing that the genomic data reflect temporal signal, so a molecular clock was estimated using a least-squares dating (LSD version 0.3) method [30] with 1000 samples for the confidence interval. The reliability of the analysis was confirmed using a permutation test in which the sampling years were randomized for 100 replications, with a Z-score test [31].

To detect signatures of natural selection in the ST2727 outbreak samples, we used a McDonald–Kreitman Test (MKT) implemented in the R package PopGenome [32]. The MKT compares the ratio of non-synonymous to synonymous mutations within strains (i.e. polymorphisms) (P_n/P_s) to the ratio of variation between strains (i.e. divergence) (D_n/D_s). A neutrality index $(P_n/P_s)/(D_n/D_s)>1$ is indicative of negative selection and >1 is characteristic of positive selection [33]. The MKT was performed for the gene and the whole genome datasets independently.

RESULTS

Clinical scenario and diagnosis

This study was initiated when outbreak status was called in an NICU due to a fatal case of a neonatal patient presenting with symptoms of respiratory and gastrointestinal infections. Despite treatment with meropenem, vancomycin, cefoperazone and sulbactam, the neonate died from early-/late-onset neonatal septicaemia, septic shock, gastrointestinal haemorrhage and multiple organ dysfunction syndrome. A blood sample from the neonate tested positive for *K. pneumoniae*. In the days after the fatal case, 20 more neonates were sampled along with an NICU staff member who presented with symptoms of infection (Fig. 1c). According to a Vitek II system (see Methods, Table S2), stool samples collected from the 21 neonates co-housed in the single-room NICU and the staff member tested positive for *K. pneumoniae*. The neonates presented with a wide range of symptoms: three with respiratory and digestive symptoms, 13 with respiratory disease such as dyspnoea, severe asphyxia, tachypnoea and/or severe cyanosis, one with digestive symptoms alone, while three presented with normal health (Table 1). The staff member presented with digestive symptoms including vomiting and severe diarrhoea. MLST classification using the genes *gapA*, *infB*, *mdh*, *pgi*, *phoE* and *rpoB* showed that 20/21 neonate isolates were novel, revealing a new MLST designation, ST2727, whereas one neonate was of ST477 and the staff member was infected with a distinct and well-characterized hypervirulent *K. pneumoniae* ST23. This suggested that the fatality was caused by the ST2727, and demonstrated that the staff member was not the cause of the event. Here, we sought to understand the carriage and genomic characteristics of ST2727 in the neonates and test the hypothesis of transmission in this closed scenario.

Genomics-based evaluation of the NICU strains

To determine the genomic characteristics of the bacteria recovered in this study, the complete genomes of all 20 ST2727 isolates were sequenced using a paired-end short read sequencing methodology. The lengths of the assembled genomes ranged from 5192865 to 5202717 bp that encoded between 4706 and 5191 annotated genes (Table S1). The GC content of all genomes was 57%. Genomic analysis showed the isolates exhibited high genomic similarity (0.018% sequence divergence), with 1066 SNVs along their genomes corresponding to an average of one SNV every 2392 bases. The variation observed among the

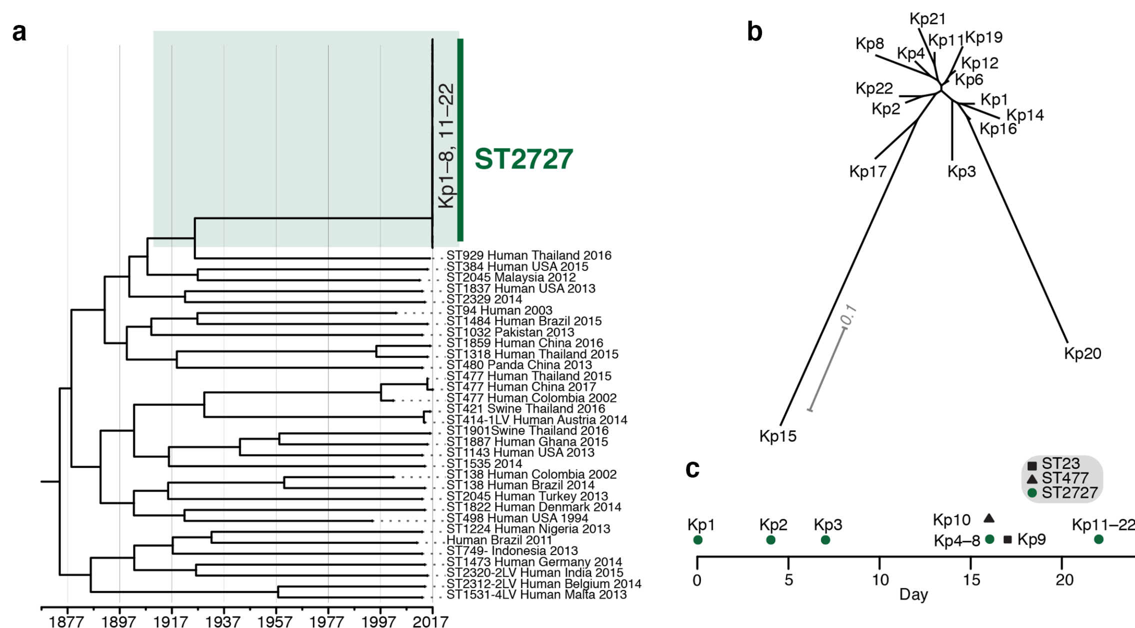

Fig. 1. Genomic epidemiology and timeline of *K. quasipneumoniae* subsp. *similipneumoniae.* (a) Evolutionary relationships and timescale of evolution of 54 *K. quasipneumoniae* subsp. *similipneumoniae* (KpIIB) strains. (b) Phylogenetic tree of the ST2727 isolates collected in the NICU during February and March 2017. The phylogenetic tree was built using the BioNJ algorithm on SplitsTree. Scale bar represents nucleotide substitutions per site. (c) Timeline of sample collection in the NICU, where calendar dates have been replaced by a timeline where the first sample was collected on day 0.

ST2727 samples was at the lower range to the median divergence of 0–0.08% observed among the same MLST types [5]. The high similarity of the ST2727 strains suggests a clonal population. Phylogenetic analysis using the SNV sites of the core genome identified weak clustering between isolates (Fig. 1b). A branch containing five isolates (Kp1, 3, 14, 16 and 20) could be distinguished from the remaining isolates, but potential transmission patterns could not be deduced due to low branch lengths between the clusters. The tip branches of Kp15 and Kp20 were considerably longer, suggesting evolution subsequent to divergence from other isolates (Fig. 1b, Table S3). Our investigation of SNVs with potential importance for resistance identified two unique polymorphisms (R124Q and T142A) in the carbohydrate porin *scrY* gene of Kp1 that require further investigation.

Phylogenies generated from a core genome of 3611 globally sampled *Klebsiella* strains (Fig. S1, Data S1), including isolates from the present study, revealed that the ST2727 strains belong to *K. quasipneumoniae* subsp. *similipneumoniae* [34] (Figs 1a and S2). A root-to-tip regression of sampling dates and genetic distance of 54 *K. quasipneumoniae* subsp. *similipneumoniae* genomes sampled during 1994–2017, including the ST2727 genomes, showed a positive correlation (correlation coefficient: 0.64) between time and mutation, indicating that the dataset contained sufficient signal for the estimation of a molecular clock. We used the least-squares dating method to estimate an evolutionary rate for the *K. quasipneumoniae* subsp. *similipneumoniae* clade of 4.596×10^{-5} nucleotide substitutions per site per year [95% confidence interval (95% CI) $4.180–4.980\times10^{-5}$]. Whilst these estimates are faster than those estimated for representative bacterial orders or families (approximately 10^{-7}) [35], our estimates are consistent with estimates made independently for *Klebsiella* clones and other bacterial clades (10^{-3} to 10^{-5}) [36, 37]. The ST2727 group exhibited a genomic divergence of >90 years [mean time to the most recent common ancestor (TMRCA) 1926; 95% CI, 1917–1933] to the most closely related isolate collected in 2016 from a patient from Thailand (KPPSTH03; MLST type ST929-1LV) (Fig. 1a).

The TMRCA of all *K. quasipneumoniae* subsp. *similipneumoniae* lineages was 1874 (95% CI, 1861–1886), whereas the mean TMRCA of ST2727 collected within the hospital was approximately 3 months prior to the first case Kp1 (mean TMRCA 2016.94, 95% CI 2016.88–2016.97). This showed a divergence time much greater than the duration of sampling across 22 days in February–March 2017 (Fig. 1c). These results suggest that what was designated as an outbreak was instead the colonization of infants by a community of bacteria that were transmitted to the neonates during this period. Due to the variation present between ST2727 isolates, it is unlikely that the colonization occurred through direct transmission between one neonate and another. We suggest instead that the isolates were introduced to the hospital or the ward in the months prior to the outbreak and were sustained within the hospital during this period. The precise mechanism of colonization of the neonates is not clear without comprehensive sampling of the built environment and the equipment used in the treatment and care of the neonates.

Table 1. Patient and *Klebsiella pneumonia* strains

Sample ID	MLST*	Patient	Birth	Symptoms†	Antibiotics	Invasive procedures	Clinical diagnosis
KP1	ST2727	Neonate	Vaginal	Digestive, respiratory	Yes	Indwelling gastric tube (IGT)	Premature, low birth weight (LBW), neonatal septicaemia, septic shock, gastrointestinal bleeding, multiple-organ failure
KP2	ST2727	Neonate	Caesarean	Digestive, respiratory	Yes	Venous catheter (VC)	Premature, LBW
KP3	ST2727	Neonate	Caesarean	Digestive, respiratory	Yes	Intubation	Premature, LBW, hyaline membrane disease, hypocalcaemia
KP4	ST2727	Neonate	Vaginal	Respiratory	No	IGT	Premature, LBW
KP5	ST2727	Neonate	Vaginal	Respiratory	No	IGT	Premature, LBW
KP6	ST2727	Neonate	Caesarean	Respiratory	Yes	IGT	Premature, LBW, congenital infection
KP7	ST2727	Neonate	Caesarean	Respiratory	Yes	VC, artery intubation (AI), artificial respirator, intubation	Premature, LBW, pneumonia
KP8	ST2727	Neonate	Caesarean	Respiratory	No	No	Premature, aspiration of amniotic fluid and meconium syndrome
KP9	ST23	Staff	N/A	Digestive	–	No data	Healthy
KP10	ST477	Neonate	Vaginal	Digestive	Yes	No	Swallowing syndrome of newborn, intestinal infection
KP11	ST2727	Neonate	Caesarean	Respiratory	Yes	VC, artificial respirator intubation	LBW, pneumonia
KP12	ST2727	Neonate	Vaginal	Respiratory	Yes	No	Premature, wet lung of the newborn
KP13	ST2727	Neonate	Caesarean	Asymptomatic	No	No	Premature, LBW, polydactyly, patent foramen ovale
KP14	ST2727	Neonate	Caesarean	Respiratory	Yes	Intubation	Premature, LBW, severe asphyxia, congenital infection
KP15	ST2727	Neonate	Caesarean	Respiratory	No	No	Premature, LBW, wet lung of the newborn
KP16	ST2727	Neonate	Vaginal	Respiratory	No	IGT, nasal catheter, VC	Premature, LBW, neonatal asphyxia, bronchial dysplasia
KP17	ST2727	Neonate	Caesarean	Respiratory	Yes	IGT	Premature, LBW, congenital infection
KP18	ST2727	Neonate	Vaginal	Non-infection	No	IGT	Premature, LBW
KP19	ST2727	Neonate	Vaginal	Asymptomatic	No	No	Premature, LBW
KP20	ST2727	Neonate	Vaginal	Respiratory	Yes	AI, venipuncture	Premature, LBW, intestinal infection
KP21	ST2727	Neonate	Caesarean	Respiratory	Yes	VC	Premature, LBW
KP22	ST2727	Neonate	Caesarean	Asymptomatic	No	No	Premature, polydactyly, patent foramen ovale

*Multilocus sequence typing.
†Symptoms are classified depending of what type of infection they are related to; 'Non-infections' suggests the patient has symptoms related to other diseases that are non-infectious, for example symptoms related to cardio-respiratory chronic diseases; and 'Asymptomatic' refers to no symptoms at all. 'Respiratory' and 'Digestive' refer to symptoms related to respiratory infections and digestive infections, respectively.

The core genome of the ST2727 clade exhibited positive selection in comparison with strains in the *K. quasipneumoniae* subsp. *similipneumoniae* clade [MKT, neutrality index (NI): 0.35, NI<1], indicating that ST2727 isolates are under selection pressure. Gene-wise estimates of selection pressure in the ST2727 clade in comparison with *K. quasipneumoniae* subsp. *similipneumoniae* strains showed that 21 genes were under negative selection (NI>1) (Table S4) and 10 were under positive selection (Table S5), whereas mutations in the remaining genes were either strongly deleterious or were under neutral selection pressure [33]. Many of these genes are fundamental to bacterial cell biology and have been functionally characterized in various *K. pneumoniae* strains, and these functions can be expected to be conserved in *K. quasipneumoniae* subsp. *similipneumoniae*. Genes under purifying or negative selection included genes coding for transcription factors, metabolic enzymes, a structural protein and proteins for active transport of solutes (Table S4). These transporter proteins that were found to be under purifying selection conform to a related set of protein families: the ATP-Binding Cassette (ABC) transporters and Major Facilitator Superfamily (MFS) transporters, as well as antiporters and symporters. ABC transporters under negative selection were those designated as responsible for the transport of thiamine, nickel and carbohydrates, which are required for bacterial survival in nutrient-rich environments [38]. MFS transporters uptake nutrients from rich environments using chemiosmotic ion gradients [38], while antiporters and symporters maintain ion homeostasis for these and other functions [38]. Taken together, this suggests that the ST2727 population has adapted for growth in a nutrient-poor environment. Conversely, while the genes under positive selection also include some for active transport of solutes (Table S5), these are transporters responsible for the export of toxins and antibiotics as efflux systems: for example, genes coding for efflux RND transporter permease subunits (drug efflux) and HlyD toxin secretion [38]. This reliance on drug efflux is also consistent with the observation that the structural protein peptidoglycan glycosyltransferase/peptidoglycan DD-transpeptidase (PBP1A) encoded by the gene *mrcA* was under negative selection: PBP1A catalyses the transglycosylation and transpeptidation of murein, which is a major component of the cell wall, and PBP1A is also the target of beta-lactam antibiotics [38].

O- and K-antigen serogroups of NICU isolates

WGS analysis using Kaptive from Kleborate [22, 39] identified that ST2727 strains have a KL55 capsular type (*wzi*56) (Table S6). O-typing of the surface lipopolysaccharide revealed all ST2727 were of the O3/O3a serotype, which has a strong adjuvant effect compared with other lipopolysaccharide types [40], but is a rarely reported serotype with no known clinical impact [41]. Genome sequence data revealed that all isolates were *wcaG*– (Fig. 2a).

Genes associated with virulence and AMR

All ST2727 isolates shared three genes considered diagnostic as virulence factors (Fig. 3a, Table S7): *uge* (uridine diphosphate galacturonate 4-epimerase), *fimH* (type 1 fimbrial adhesion) and *mrkD* (type 3 fimbrial adhesion). The gene *uge* is responsible for the conversion of UDP-GlcA to UDP-GalA and is key for the production of polysaccharides containing GalA residues, a feature which has been shown to promote colonization of human tissues [42]. Fimbriae are an important feature of *Klebsiella*, and the gene *fimH* encodes a well-conserved adhesin subunit that is found in around 90% of *Klebsiella* strains [43]. This gene has high mobility within clones through horizontal transfer [43]. The gene encoding *mrkD* is associated with 'type III' fimbriae, important for adhesion to promote establishment of bacterial biofilms in harsh environments [44].

Antimicrobial susceptibility testing using minimal inhibitory concentration (MIC) analysis indicated that all isolates were sensitive to all third-generation cephalosporins tested (Table S2). In terms of other AMR, two genes encoding beta-lactamases were detected (Fig. 2a, Table S7): the ubiquitous *ampH*, responsible for resistance to penicillin G, cefoxitin and cephalosporin C; and $bla_{\text{OKP-B-10}}$, which is a diagnostic feature of *K. quasipneumoniae* subsp. *similipneumoniae* [45].

Secretion systems

Protein secretion systems are key components of virulence in many bacterial pathogens [46], and in *Klebsiella* the T2SS is the best characterized of these virulence determinants [47]. We identified a unique T2SS architecture in *K. quasipneumoniae* subsp. *similipneumoniae*. All ST2727 isolates collected have a similar T2SS architecture, typically composed of *pulS* and *pulA-pulM* [48] (Fig. 3b), however a clear homologue of GspN was absent in *K. quasipneumoniae* subsp. *similipneumoniae* genomes; GspN is a bitopic, integral membrane protein present in the inner membrane embedded section of the core T2SS in *K. pneumoniae* and *K. oxytoca* [49], essential for protein secretion in *K. pneumoniae* but not in *K. oxytoca* [50]. Other common secretion systems (T3SS, T4SS, T5SS and T6SS) were not found in any of the ST2727 isolates, while HlyD, a diagnostic component of the type 1 secretion system (T1SS), was detected in the ST2727 strains (Table S5).

Evolutionary relationship of ST2727 to globally isolated *Klebsiella* strains

Klebsiella quasipneumoniae was previously considered of low clinical risk [3–5]. Mapping of virulence and resistance phenotypes observed among *K. quasipneumoniae* subsp. *similipneumoniae* and globally collected *Klebsiella* genomes showed that two *K. quasipneumoniae* subsp. *similipneumoniae* MLSTs, ST1901 (GCF_002248055.1) and ST2320 (GCF_001729665.1), and a *K. variicola* type (ST981) (GCF_001463685.1) [51] contained virulence markers that were previously only observed among *K. pneumoniae* strains [22, 23]. The ST1910 genome collected in Thailand in 2016

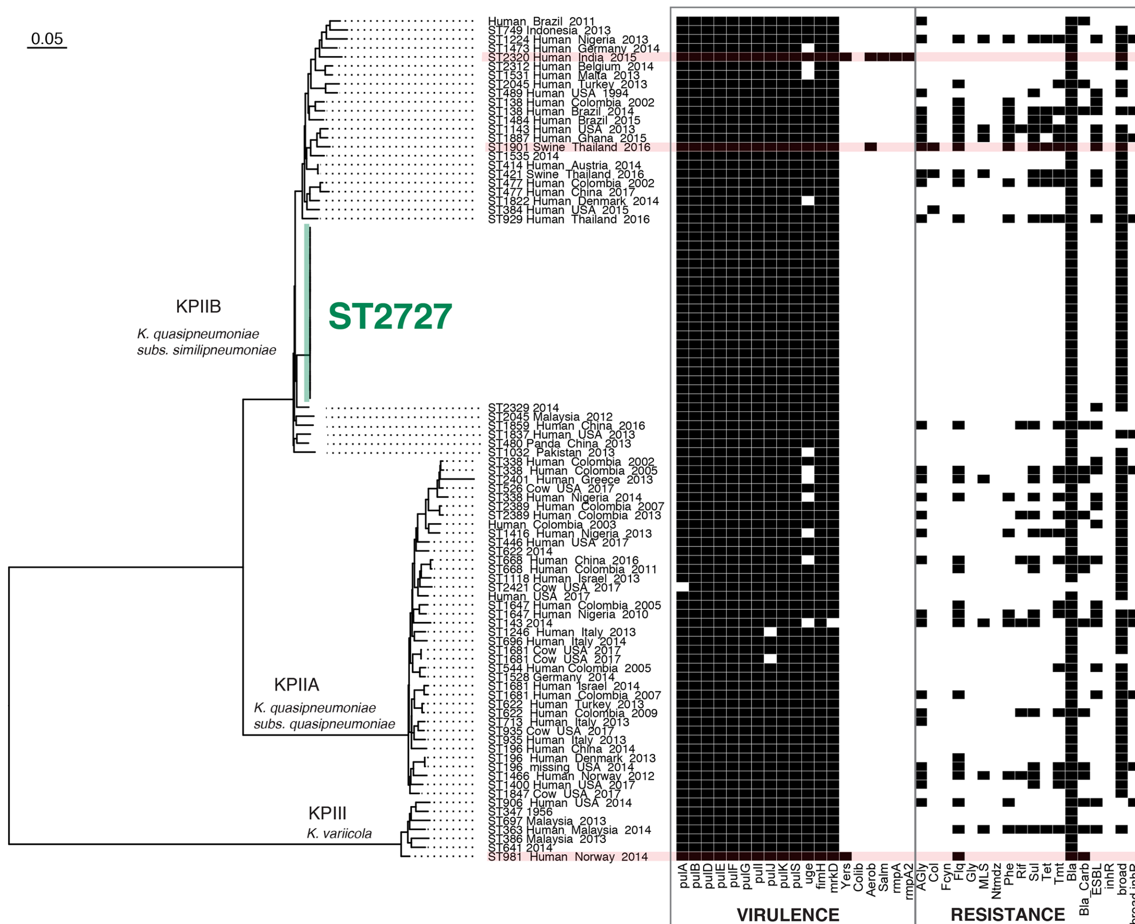

Fig. 2. Phylogenetic distribution of virulence and resistance genes among KPII and KPIII genomes. The presence of virulent and resistance genes is shown in black. Virulent genes are grouped in categories: T2SS (*pulA*, *pulB*, *pulD*, *pulE*, *pulF*, *pulG*, *pulI*, *pulJ*, *pulK*, *pulS*) adhesion genes (*uge*, *fimH*, *mrkD*), Yers (yersiniabactin), Coli (colibactin), Aerob (aerobactin), Salmo (Salmochelin) and *rmpA, rmpA_2* (hypermucoid). Resistant genes are grouped in categories: Gly (glycopeptides), MLS (macrolides), Phe (phenicols), Rif (rifampin), Sul (sulphonamides), Tet (tetracyclines), Tmt (trimethoprim), Bla (beta-lactamases), Bla_Carb (carbapenemase), ESBL (extended spectrum beta-lactamases), inhR (extended spectrum beta-lactamases with resistance to beta-lactamase inhibitors), broad (broad spectrum beta-lactamases), broad.inhR (broad spectrum beta-lactamases with resistance to beta-lactamase inhibitors). Virulent MLST types are highlighted in red.

contained the virulence marker aerobactin (*iuc3*), whereas the ST2320 strain collected in India during 2015 can express aerobactin (*iuc1*), yerseniabactin (*ybt 9*; *ICEKp3*) and salmochelin (*iro1*), as well as producing a hypermucoidy phenotype (*rmpA_4, rmpA2_3*). The emergence of virulence markers among *K. quasipneumoniae* genomes raises concerns as there has been a parallel increase in hospital-acquired infections caused by *K. quasipneumoniae* [5, 7]. Figure 4 shows that ST2727 is closely related to ST498, a group that has been found to have numerous AMR genes including *aadB*, *sul1* and *blaOKP-B-7*, and the genes *blaOXA-2* and *blaSHV-18* that encode extended-spectrum beta-lactamases [8] (Fig. 2).

DISCUSSION

Colonization of the neonate gut is a complex process with contributions from the mother's microbiome and environmental sources, which cannot easily be disentangled [52–54]. In this study, 21 neonates housed in a single room were sampled across 22 days for the carriage of *Klebsiella* in response to an outbreak-status event. The infection control procedures required that an outbreak be called in response to multiple cases of infection being reported in the same ward within a short period. These infections were diagnosed on clinical grounds, suspected to be hospital-acquired, and triggered the need for sampling and detailed diagnosis of other potentially at-risk neonatal patients. However, it is now clear from genomics data that the hyper-virulent ST23 isolated from the staff member was an irrelevant coincidence to the sepsis event in the neonates. A major outcome from this retrospective, deep, sequence analysis is the data that it provides on the colonization of the neonates by a strain of *K. quasipneumoniae* subsp. *similipneumoniae* from the NICU environment.

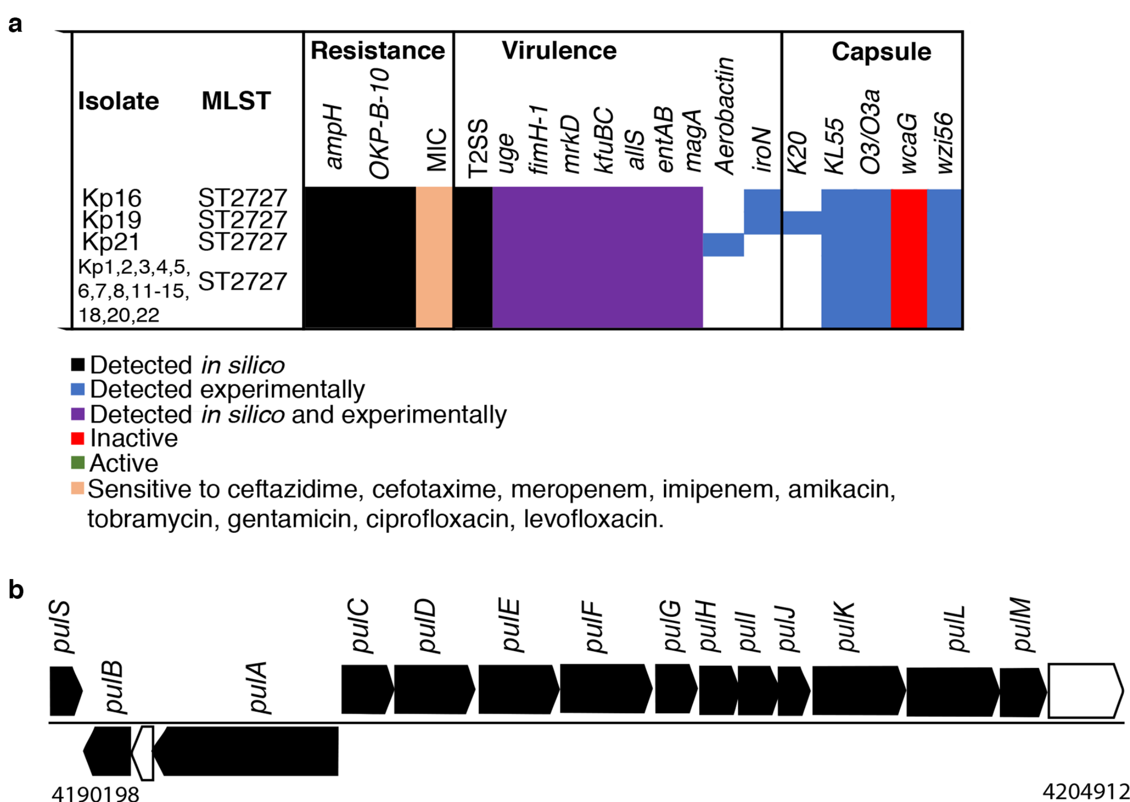

Fig. 3. Major resistance, virulence and capsular genes detected in ST2727. (a) Summary of genes contributing to drug resistance, iron acquisition and other virulence-enhancing features. MIC, minimal inhibitory concentration. (b) Genomic architecture of the T2SS cassette in ST2727 andother *K. quasipneumoniae* subsp. *similipneumoniae*. Gene predictions arecolor-coded according to the legend, with black representing genes detected *insilico* with greater than 80% sequence identity to the corresponding genesin *K. pneumoniae*.

The *Klebsiella* isolated from the sick neonates carried a previously unreported sequence type (ST2727), with core-genome phylogenies showing they belonged to *K. quasipneumoniae* subsp. *similipneumoniae* [34]. Despite the availability of few *K. quasipneumoniae* genomes, our analysis of 54 available genomes including the 20 new ST2727 samples, showed positive correlation between genetic distances and date of sampling [29]. This indicates that our dataset was adequate to show that the NICU samples had diverged months prior to the detection of the first case, and showing that ST2727 was not transmitted between patients during the outbreak. These results were further supported by a permutation test where the sampling dates were randomized [31, 55]. Furthermore, the lower sampling of *K. quasipneumoniae* genomes, as compared to the greatly sampled *K. pneumoniae* genomes, would not have an effect on the estimation of the molecular clock for the outbreak samples because bacterial phylogenies in general show a large genetic distance between MLST types – signified by the long phylogenetic branch lengths between MLST types (star-shaped species-level phylogeny), indicating that adequate sampling of closely related bacteria would improve estimates rather than greater sampling of highly divergent genomes within a bacterial species. Without a detailed analysis focused on the built environments of the NICU and surrounding community it is difficult to infer with more confidence from where they originated. Overall, however, this study suggests that a population of ST2727 with some sequence variability is probably endemic within the hospital environment, and that distinct members within this population have infected the neonates in their first days of life.

It is probable that ST2727 is under antibiotic selective pressure due to their prevalence in the hospital environment for the months prior to the events analysed here. This is further confirmed with an MKT indicating positive selective pressure ongoing for genes related to efflux systems that are a key part for the transport of toxic substances as antibiotics. However, further investigation on the evolutionary mechanism selecting for resistant genes and genes related to resistance mechanisms would need to be done in order to assert these findings. Previously, estimates of selection pressure on the genes encoding beta-lactamases in the clades KpI, KpII and KpIII have shown neutral selection of these genes [51]. In agreement with this, we did not find either of the genes encoding beta-lactamases in ST2727 to be under natural selective pressure.

Although it is now highly unlikely, the data in this study cannot rule out a maternal contribution to *Klebsiella* carriage in the neonates, but we note that (i) 12/20 neonates were delivered via Caesarian section (Table 1) where the maternal contribution to the microflora should be

inconsistent and minimal, and that (ii) ST2727 is probably not a dominant sequence type in the community as it has not been sampled previously, despite the hospital having more than 1500 *Klebsiella* isolates collected from adult patients over 15 years. We suggest that a substantial contribution to carriage comes from the endemic microflora in the NICU, with this specific built environment colonized by a population corresponding to ST2727. A suggestion for future analyses would be inclusion of more detailed samplings of the NICU environment, including the built environment itself and systematic sampling of staff members and visitors. Our study also urges the continual monitoring of hospital environments to improve understanding of the dynamics of bacterial communities in hospitals as reservoirs of resistance and virulent genes [56].

While *K. quasipneumoniae* subsp. *similipneumoniae* has been less widely reported and discussed than *K. pneumoniae*, similar to our investigation, there has been an increased recognition of *K. quasipneumoniae* subspecies in hospital surveys and their association with resistance genes [57, 58], indicating that a comprehensive assessment of their role in AMR ecology and contribution to disease burden is required. Their under-recognition is also due to the mis-assignment of non-*K. pneumoniae* strains to the type species.

Previous analysis estimated that at least half of *Klebsiella* infections result from the patients' own microbiota [59]. That the neonate who died probably had a bowel perforation to initiate sepsis from a strain carried in the gut supports the contention that *K. quasipneumoniae* carriage contributes to the risk of infection in hospital environments. Thus, carriage of drug-resistant or hypervirulent clones of *Klebsiella*, including *K. quasipneumoniae,* in the gut would be of great concern in people with lowered immune systems – such as neonates – or those with conditions requiring surgical treatment where bowel perforation is a risk factor [52]. Our study highlights the importance of WGS for elucidating the transmission and carriage of hospital-resident bacterial populations, and urges for the early identification and eradication of persistent strains that increase the rate of hospital-acquired infections.

Funding information

This work was supported by research grants from the National Natural Science Foundation of China (no. 81171614), the Health Department of Zhejiang Province of the People's Republic of China (no. 2011KYA106), the Zhejiang Provincial Programme for the Cultivation of High-level Innovative Health Talents (no. 2012, 241) and Programme Grant 1092262 from the National Health and Medical Research Council, Australia.

Acknowledgements

We thank Dr Jiangning Song for his input in the early stages of this analysis.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).

References

1. Wyres KL, Holt KE. *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Curr Opin Microbiol* 2018;45:131–139.
2. Zhan L, Wang S, Guo Y, Jin Y, Duan J *et al*. Outbreak by Hypermucoviscous *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in a tertiary hospital in China. *Front Cell Infect Microbiol* 2017;7.
3. Long SW, Linson SE, Ojeda Saavedra M, Cantu C, Davis JJ *et al*. Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*. *mSphere* 2017;2.
4. Long SW, Olsen RJ, Eagar TN, Beres SB, Zhao P *et al*. Population genomic analysis of 1777 extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* Isolates, Houston, Texas: unexpected abundance of clonal group 307. *mBio* 2017;8:e00489-17.
5. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA *et al*. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc Natl Acad Sci U S A* 2015;112:E3574–E3581.
6. Blin C, Passet V, Touchon M, Rocha EPC, Brisse S. Metabolic diversity of the emerging pathogenic lineages of *Klebsiella pneumoniae*. *Environ Microbiol* 2017;19:1881–1898.
7. Ejaz H, Wang N, Wilksch JJ, Page AJ, Cao H *et al*. Phylogenetic analysis of *Klebsiella pneumoniae* from hospitalized children, Pakistan. *Emerg Infect Dis* 2017;23:1872–1875.
8. 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:e00438-16.
9. Maatallah M, Vading M, Kabir MH, Bakhrouf A, Kalin M *et al*. Klebsiella variicola is a frequent cause of bloodstream infection in the Stockholm area, and associated with higher mortality compared to K. pneumoniae. *PLoS One* 2014;9:e113539.
10. Nicolás MF, Ramos PIP, Marques de Carvalho F, Camargo DRA, de Fátima Morais Alves C *et al*. Comparative genomic analysis of a clinical isolate of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, a KPC-2 and OKP-B-6 Beta-lactamases producer harboring two drug-resistance plasmids from Southeast Brazil. *Front Microbiol* 2018;9:220.
11. Rodrigues C, Passet V, Rakotondrasoa A, Brisse S. Identification of *Klebsiella pneumoniae, Klebsiella quasipneumoniae, Klebsiella variicola* and related phylogroups by MALDI-TOF mass spectrometry. *Front Microbiol* 2018;9:3000.
12. Lowe C, Willey B, O'Shaughnessy A, Lee W, Lum M *et al*. Outbreak of extended-spectrum β-lactamase-producing *Klebsiella oxytoca* infections associated with contaminated handwashing sinks(1). *Emerg Infect Dis* 2012;18:1242–1247.
13. Chang FY, Chou MY. Comparison of pyogenic liver abscesses caused by *Klebsiella pneumoniae* and non-*K. pneumoniae* pathogens. *J Formos Med Assoc* 1995;94:232–237.
14. Gu D, Dong N, Zheng Z, Lin D, Huang M *et al*. A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. *Lancet Infect Dis* 2018;18:37–46.
15. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233–2239.
16. 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.
17. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
18. Bosi E, Donati B, Galardini M, Brunetti S, Sagot M-F *et al*. Medusa: a multi-draft based scaffolder. *Bioinformatics* 2015;31:2443–2451.

19. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 2010;5:e11147.
20. Boratyn GM, Thierry-Mieg J, Thierry-Mieg D, Busby B, Madden TL. Magic-BLAST, an accurate RNA-seq aligner for long and short reads. *BMC Bioinformatics* 2019;20:405.
21. Treangen TJ, Ondov BD, Koren S, Phillippy AM. The harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol* 2014;15:524.
22. Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R *et al*. Identification of *Klebsiella* capsule synthesis loci from whole genome data. *Microb Genom* 2016;2:e000102.
23. Lam MMC, Wyres KL, Duchêne S, Wick RR, Judd LM *et al*. Population genomics of hypervirulent Klebsiella pneumoniae clonal-group 23 reveals early emergence and rapid global dissemination. *Nat Commun* 2018;9:2703.
24. Li J, Tai C, Deng Z, Zhong W, He Y *et al*. VRprofile: gene-cluster-detection-based profiling of virulence and antibiotic resistance traits encoded within genome sequences of pathogenic bacteria. *Brief Bioinform* 2018;19:566–574.
25. Dunstan RA, Heinz E, Wijeyewickrema LC, Pike RN, Purcell AW *et al*. Assembly of the type II secretion system such as found in Vibrio cholerae depends on the novel Pilotin AspS. *PLoS Pathog* 2013;9:e1003117.
26. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S *et al*. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015;31:3691–3693.
27. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
28. Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* 2006;23:254–267.
29. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol* 2016;2:vew007.
30. To T-H, Jung M, Lycett S, Gascuel O. Fast dating using least-squares criteria and algorithms. *Syst Biol* 2016;65:82–97.
31. Duchene S, Duchene DA, Geoghegan JL, Dyson ZA, Hawkey J *et al*. Inferring demographic parameters in bacterial genomic data using Bayesian and hybrid phylogenetic methods. *BMC Evol Biol* 2018;18:1–11.
32. Pfeifer B. An introduction to the PopGenome package. *Cran-R* 2015:1–18.
33. Stoletzki N, Eyre-Walker A. Estimation of the neutrality index. *Mol Biol Evol* 2011;28:63–70.
34. Brisse S, Passet V, Grimont PAD. Description of *Klebsiella quasipneumoniae* sp. nov., isolated from human infections, with two subspecies, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* subsp. nov. and *Klebsiella quasipneumoniae* subsp. *similipneumoniae* subsp. nov., and demonstration that *Klebsiella singaporensis* is a junior heterotypic synonym of *Klebsiella variicola*. *Int J Syst Evol Microbiol* 2014;64:3146–3152.
35. Sanjuán R, Nebot MR, Chirico N, Mansky LM, Belshaw R. Viral mutation rates. *J Virol* 2010;84:9733–9748.
36. Cella E, Ciccozzi M, Lo Presti A, Fogolari M, Azarian T *et al*. Multi-drug resistant *Klebsiella pneumoniae* strains circulating in hospital setting: whole-genome sequencing and Bayesian phylogenetic analysis for outbreak investigations. *Sci Rep* 2017;7:3534.
37. Duchêne S, Holt KE, Weill F-X, Le Hello S, Hawkey J *et al*. Genome-scale rates of evolutionary change in bacteria. *Microb Genom* 2016;2:e000094.
38. Mitchell AL, Attwood TK, Babbitt PC, Blum M, Bork P *et al*. InterPro in 2019: improving coverage, classification and access to protein sequence annotations. nucleic acids Res 2019;47:D351–D60.
39. Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. *J Clin Microbiol* 2018;56.
40. Kato N, Kido N, Ohta M, Naito S, Nakashima I. Adjuvant activity of *Klebsiella* O3 lipopolysaccharide: comparative study using defined uniform salt forms. *Microbiol Immunol* 1984;28:659–666.
41. Rollenske T, Szijarto V, Lukasiewicz J, Guachalla LM, Stojkovic K *et al*. Cross-specificity of protective human antibodies against *Klebsiella pneumoniae* LPS O-antigen. *Nat Immunol* 2018;19:617–624.
42. Regué M, Hita B, Piqué N, Izquierdo L, Merino S *et al*. A gene, uge, is essential for *Klebsiella pneumoniae* virulence. *Infect Immun* 2004;72:54–61.
43. Stahlhut SG, Chattopadhyay S, Struve C, Weissman SJ, Aprikian P *et al*. Population variability of the FimH type 1 fimbrial adhesin in *Klebsiella pneumoniae*. *J Bacteriol* 2009;191:1941–1950.
44. Wilksch JJ, Yang J, Clements A, Gabbe JL, Short KR *et al*. MrkH, a novel c-di-GMP-dependent transcriptional activator, controls *Klebsiella pneumoniae* biofilm formation by regulating type 3 fimbriae expression. *PLoS Pathog* 2011;7:e1002204.
45. Henderson TA, Young KD, Denome SA, Elf PK. Ampc and AMPH, proteins related to the class C beta-lactamases, bind penicillin and contribute to the normal morphology of *Escherichia coli*. *J Bacteriol* 1997;179:6112–6121.
46. Gunasinghe SD, Webb CT, Elgass KD, Hay ID, Lithgow T. Super-resolution imaging of protein secretion systems and the cell surface of gram-negative bacteria. *Front Cell Infect Microbiol* 2017;7:220.
47. Cianciotto NP, White RC. Expanding role of type II secretion in bacterial pathogenesis and beyond. *Infect Immun* 2017;85:1–17.
48. Nivaskumar M, Francetic O. Type II secretion system: a magic beanstalk or a protein escalator. *Biochim Biophys Acta* 2014;1843:1568–1577.
49. Chernyatina AA, Low HH. Core architecture of a bacterial type II secretion system. *Nat Commun* 2019;10:5437.
50. Filloux A. The underlying mechanisms of type II protein secretion. *Biochim Biophys Acta* 2004;1694:163–179.
51. Samuelsen Ørjan, Overballe-Petersen S, Bjørnholt JV, Brisse S, Doumith M *et al*. Molecular and epidemiological characterization of carbapenemase-producing *Enterobacteriaceae* in Norway, 2007 to 2014. *PLoS One* 2017;12:e0187832.
52. Raveh-Sadka T, Thomas BC, Singh A, Firek B, Brooks B *et al*. Gut bacteria are rarely shared by co-hospitalized premature infants, regardless of necrotizing enterocolitis development. *elife* 2015;4.
53. Brooks B, Olm MR, Firek BA, Baker R, Thomas BC *et al*. Strain-resolved analysis of hospital rooms and infants reveals overlap between the human and room microbiome. *Nat Commun* 2017;8:1814.
54. Yassour M, Jason E, Hogstrom LJ, Arthur TD, Tripathi S *et al*. Strain-Level analysis of mother-to-child bacterial transmission during the first few months of life. *Cell Host Microbe* 2018;24:146–154.
55. Mir D, Jung M, Delatorre E, Vidal N, Peeters M *et al*. Phylodynamics of the major HIV-1 CRF02_AG African lineages and its global dissemination. *Infect Genet Evol* 2016;46:190–199.
56. Chng KR, Li C, Bertrand D, Ng AHQ, Kwah JS *et al*. Cartography of opportunistic pathogens and antibiotic resistance genes in a tertiary hospital environment. *Nat Med* 2020;26:941–951.
57. Imai K, Ishibashi N, Kodana M, Tarumoto N, Sakai J *et al*. Clinical characteristics in blood stream infections caused by *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*: a comparative study, Japan, 2014-2017. *BMC Infect Dis* 2019;19:946.
58. Mathers AJ, Crook D, Vaughan A, Barry KE, Vegesana K *et al*. *Klebsiella quasipneumoniae* provides a window into carbapenemase gene transfer, plasmid rearrangements, and patient interactions with the hospital environment. *Antimicrob Agents Chemother* 2019;63:1–12.
59. Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR *et al*. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. *Clin Infect Dis* 2017;65:208–215.