

# Investigation of sequential outbreaks of *Burkholderia cepacia* and multidrug-resistant extended spectrum $\beta$ -lactamase producing *Klebsiella* species in a West African tertiary hospital neonatal unit: a retrospective genomic analysis



Uduak Okomo, Madikay Senghore\*, Saffiatou Darboe\*, Ebrima Bojang\*, Syed M A Zaman, Mohammad Jahangir Hossain, Davis Nwakanma, Kirsty Le Doare, Kathryn E Holt, Nina Judith Hos, Joy E Lawn, Stephen D Bentley, Beate Kampmann



## Summary

**Background** Sick newborns admitted to neonatal units in low-resource settings are at an increased risk of developing hospital-acquired infections due to poor clinical care practices. Clusters of infection, due to the same species, with a consistent antibiotic resistance profile, and in the same ward over a short period of time might be indicative of an outbreak. We used whole-genome sequencing (WGS) to define the transmission pathways and characterise two distinct outbreaks of neonatal bacteraemia in a west African neonatal unit.

**Methods** We studied two outbreaks of *Burkholderia cepacia* and multidrug-resistant extended spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae* in a neonatal unit that provides non-intensive care on the neonatal ward in the Edward Francis Small Teaching Hospital, Banjul, The Gambia. We used WGS to validate and expand findings from the outbreak investigation. We retrospectively sequenced all clinical isolates associated with each outbreak, including isolates obtained from swabs of ward surfaces, environmental fluid cultures, intravenous fluids, and antibiotics administered to newborns. We also sequenced historical *B cepacia* isolates associated with neonatal sepsis in the same ward.

**Results** Between March 1 and Dec 31, 2016, 321 blood cultures were done, of which 178 (55%) were positive with a clinically significant isolate. 49 episodes of neonatal *B cepacia* bacteraemia and 45 episodes of bacteraemia due to ESBL-producing *K pneumoniae* were reported. WGS revealed the suspected *K pneumoniae* outbreak to be contemporaneous outbreaks of *K pneumoniae* (ST39) and previously unreported *Klebsiella quasipneumoniae* subspecies *similipneumoniae* (ST1535). Genomic analysis showed near-identical strain clusters for each of the three outbreak pathogens, consistent with transmission within the neonatal ward from extrinsically contaminated in-use intravenous fluids and antibiotics. Time-dated phylogeny, including retrospective analysis of archived bacterial strains, suggest *B cepacia* has been endemic in the neonatal ward over several years, with the *Klebsiella* species a more recent introduction.

**Interpretation** Our study highlights the emerging threat of previously unreported strains of multidrug-resistant *Klebsiella* species in this neonatal unit. Genome-based surveillance studies can improve identification of circulating pathogen strains, characterisation of antimicrobial resistance, and help understand probable infection acquisition routes during outbreaks in newborn units in low-resource settings. Our data provide evidence for the need to regularly monitor endemic transmission of bacteria within the hospital setting, identify the introduction of resistant strains from the community, and improve clinical practices to reduce or prevent the spread of infection and resistance.

**Funding** Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Fajara, The Gambia.

**Copyright** © 2020 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY-NC-ND 4.0 license.

## Introduction

Globally, in 2018, an estimated 2.5 million newborns died within the first 28 days of birth, and almost 80% of these deaths occurred in south Asia and sub-Saharan Africa.<sup>1</sup> Infections (especially sepsis, meningitis, and pneumonia) were among the leading causes of these deaths.<sup>2</sup> The risk of hospital-acquired infection is particularly high for newborns admitted to hospital in

low-resource settings, and is associated with overcrowding and understaffing, as well as weak infection control protocols.<sup>3</sup> Gram-negative bacterial infections are increasingly common in neonatal units, especially multidrug-resistant *Klebsiella* spp, which are implicated in outbreaks.<sup>4</sup> *Burkholderia cepacia* complex organisms are also associated with hospital outbreaks but less commonly reported in sub-Saharan Africa. Members of

*Lancet Microbe* 2020;  
1: e119–29

See [Comment](#) page e95

\*Contributed equally

Vaccines and Immunity Theme (U Okomo PhD, M Senghore PhD, S Darboe MSc, E Bojang MRes, Prof B Kampmann PhD), and Disease Control and Elimination Theme (M J Hossain MBBS, D Nwakanma PhD), Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Fajara, The Gambia; Center for Communicable Disease Dynamics, Department of Epidemiology, Harvard T H Chan School of Public Health, Boston, MA, USA (M Senghore); Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, Birmingham, UK (E Bojang); Liverpool School of Tropical Medicine, Liverpool, UK (S M A Zaman PhD); Institute of Infection and Immunity, St George's University of London, Cranmer Terrace, London, UK (K Le Doare PhD); The Vaccine Centre, Department of Clinical Research, Faculty of Infectious and Tropical Diseases (Prof K E Holt PhD, N J Hos MD, Prof B Kampmann), and Maternal, Adolescent, Reproductive, & Child Health Centre (Prof J E Lawn FMedSci), London School of Hygiene & Tropical Medicine, London, UK; Department of Infectious Diseases, Central Clinical School, Monash University, Melbourne, VIC, Australia (Prof K E Holt); Parasites and Microbes Programme, Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK (S D Bentley PhD); Department of Pathology, University of Cambridge, Cambridge, UK (S D Bentley);

and Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK (S D Bentley)

Correspondence to: Dr Uduak Okomo, Vaccines and Immunity Theme, Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine, Fajara, Banjul, The Gambia uokomo@mrc.gm

## Research in context

### Evidence before this study

Hospital-acquired infections are a major burden and safety issue for newborns globally, and particularly in low-resource settings, where they are responsible for up to half of all neonatal deaths among hospital-born babies. In such settings, hospital-acquired infections are endemic across all levels of neonatal care—both intensive and non-intensive—and relate to poor standards of care. Apart from endemic infections, outbreaks of bacterial, viral, and fungal infections are also often reported. Extended spectrum  $\beta$ -lactamase-producing Enterobacteriaceae (notably *Klebsiella pneumoniae*), *Staphylococcus aureus* (meticillin resistant and meticillin sensitive), *Serratia* spp, *Acinetobacter* spp, and *Enterobacter* spp have emerged as the major bacterial pathogens implicated in outbreaks of infection in neonatal units of developing countries. Whole-genome sequencing (WGS) is increasingly used to complement traditional epidemiological surveillance for outbreak monitoring.

We searched OVID (appendix p 2) and PubMed (appendix p 3) databases up to Feb 3, 2020, without date or language restrictions, with the search terms [neonat\* OR newborn\*] AND [infection\* OR sepsis OR septic\* OR “nosocomial infection”] along with [center(s) OR unit(s) OR nursery OR nurseries OR hospital(s) OR NICU] AND [outbreak\* OR epidemic\* OR cluster\*] AND [“whole genome sequencing”]. This search strategy identified 35 studies that reported the use of WGS to investigate neonatal outbreaks, only one of which was from sub-Saharan Africa. This study reported the identification of multidrug-resistant New Delhi metallo- $\beta$ -lactamase (NDM-5)-containing *Klebsiella quasipneumoniae* subspecies *similipneumoniae* from isolates recovered during a neonatal bacteraemia outbreak at a tertiary hospital in Nigeria in 2016,

and surveillance isolates from 2013 from the same hospital. The prevalence of NDM-5 in *Klebsiella* spp had been limited to *K pneumoniae*, with only one isolate collected from Africa.

### Added value of this study

Our phylogenetic data demonstrated the endemicity of *Burkholderia cepacia* in the neonatal ward up to 8 years before the outbreak, suggesting that there were additional, undiscovered cases before the onset of microbiological screening, and that the spread of the pathogen remained undetected over several years. Our data also show that the second outbreak previously thought to be solely due to multidrug-resistant *Klebsiella pneumoniae*, was actually simultaneous, but distinct outbreaks due to *K quasipneumoniae* subsp *similipneumoniae* and *K pneumoniae*. Our findings show, for the first time in sub-Saharan Africa, the presence of the virulence factor yersiniabactin in *K quasipneumoniae* subsp *similipneumoniae*.

### Implications of all the available evidence

Correct classification of neonatal infections as nosocomial outbreaks, and not community-acquired infections, is important to inform successful infection prevention and control strategies. These data highlight the need for precise identification of bacterial species, molecular characterisation of antimicrobial resistance, and regular microbiological surveillance in the hospital environment to detect both endemic infections and independent introduction of pathogens from the community. Infection control policies to combat hospital-acquired infections should also delineate the relative contributions of within-hospital transmission of resistant bacterial pathogens, and the introduction of resistant strains from the community.

the *B cepacia* complex are opportunistic gram-negative nosocomial pathogens that can cause serious infections in immunocompromised patients and newborns. Often found in liquid reservoirs, they can survive for long periods in water or disinfectants, and are intrinsically resistant to several antimicrobial agents. Identifying an outbreak in a neonatal unit can be challenging, but one indicator is when multiple infants are infected by the same species with a consistent antibiotic resistance profile over a short period.

In high-income settings, whole-genome sequencing (WGS) is now the reference tool for investigating bacterial transmission patterns and characterising outbreaks; it provides the resolving power to disprove transmission events indicated by conventional methods (such as antibiotic resistance profile), and can also reveal unsuspected strain clusters consistent with nosocomial transmission.<sup>5–7</sup> WGS can also be coupled with temporal and other epidemiological information to model transmission pathways and identify outbreaks.<sup>8</sup> These approaches have been applied in neonatal intensive and non-intensive units in the USA<sup>9</sup> and Europe,<sup>10</sup> but there

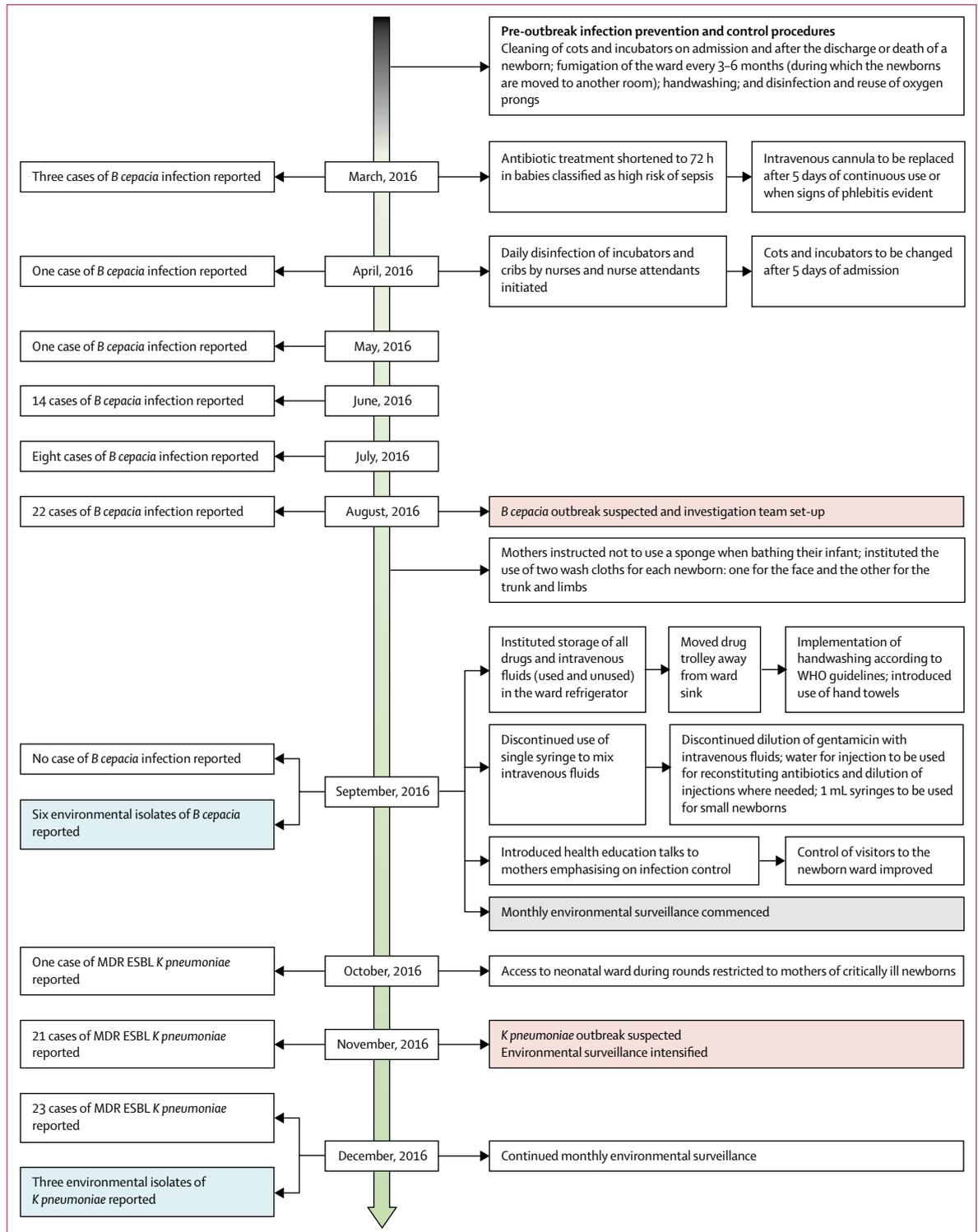
is paucity of genome-based studies from sub-Saharan Africa.<sup>11</sup> We describe two distinct outbreaks of *B cepacia* and extended spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae* bacteraemia occurring between March and December, 2016, in The Gambia's largest hospital and only neonatal unit. We did a retrospective genomic analysis to understand the phylogenetic relationships between isolates in each outbreak and to detect the presence of resistance genes and plasmids.

## Methods

### Setting

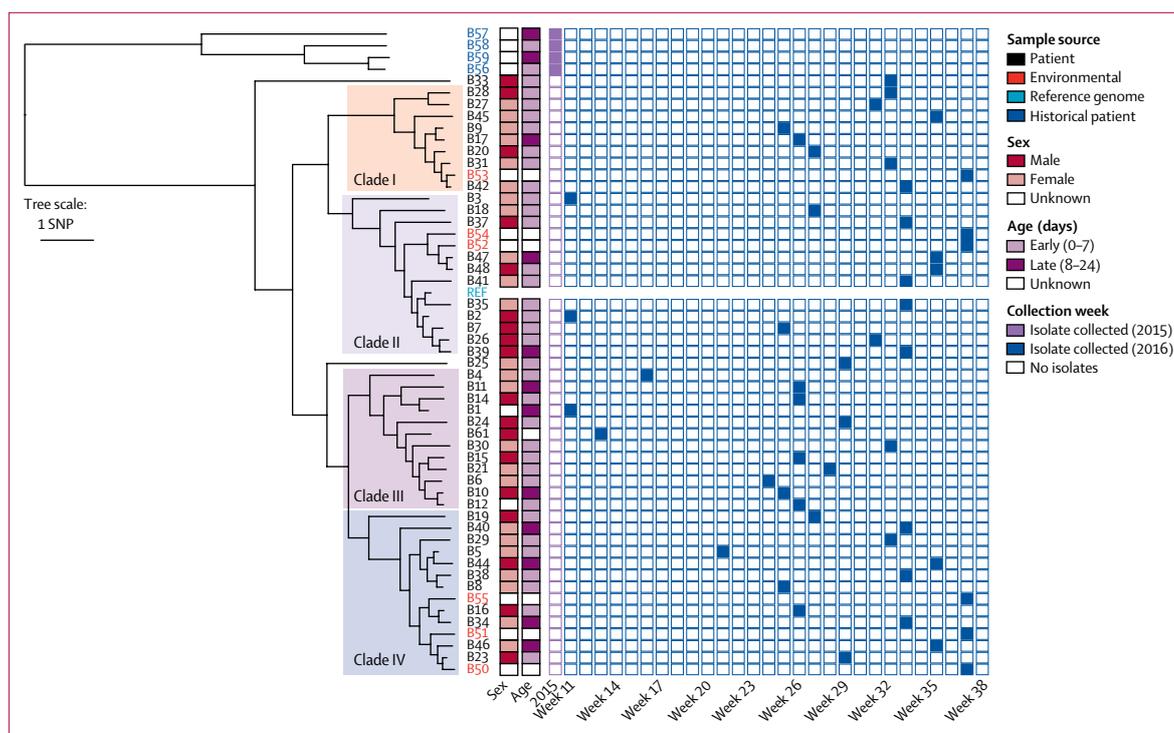
This study was done in the neonatal unit of the Edward Francis Small Teaching Hospital (EFSTH), Banjul, The Gambia—a tertiary referral hospital with 640 beds and about 6000 births each year. Annually, approximately 1400 neonates are admitted to the non-intensive care neonatal unit, which has 31 cots and four incubators.<sup>12</sup> During peak admission periods, the unit is highly congested, with cots arranged in close proximity, often with multiple neonates sharing Resuscitaires (Dräger, Lübeck, Germany) and incubators.





**Figure 1: Timing of reported *Burkholderia cepacia* and of MDR ESBL *Klebsiella pneumoniae* outbreak cases**

The outbreaks occurred at the Edward Francis Small Teaching Hospital (Banjul, The Gambia) neonatal ward; infection prevention and control procedures undertaken between March and December, 2016, are shown with subsequent environmental surveillance and control measures instituted in response to the identified *B. cepacia* and *K. pneumoniae* cases. MDR ESBL=multidrug-resistant extended spectrum  $\beta$ -lactamase.



**Figure 2:** A time-dated phylogeny of all *Burkholderia cepacia* isolates annotated with patient age, sex, and a timeline of when isolates were collected. Isolate generic identifications are shown and coloured by source: patient, environmental, historical, or reference genome. Where applicable, sex is shown, and age is given as a categorical variable of early onset (0–7 days) and late onset (8–27 days). SNP=single nucleotide polymorphism.

the Article. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

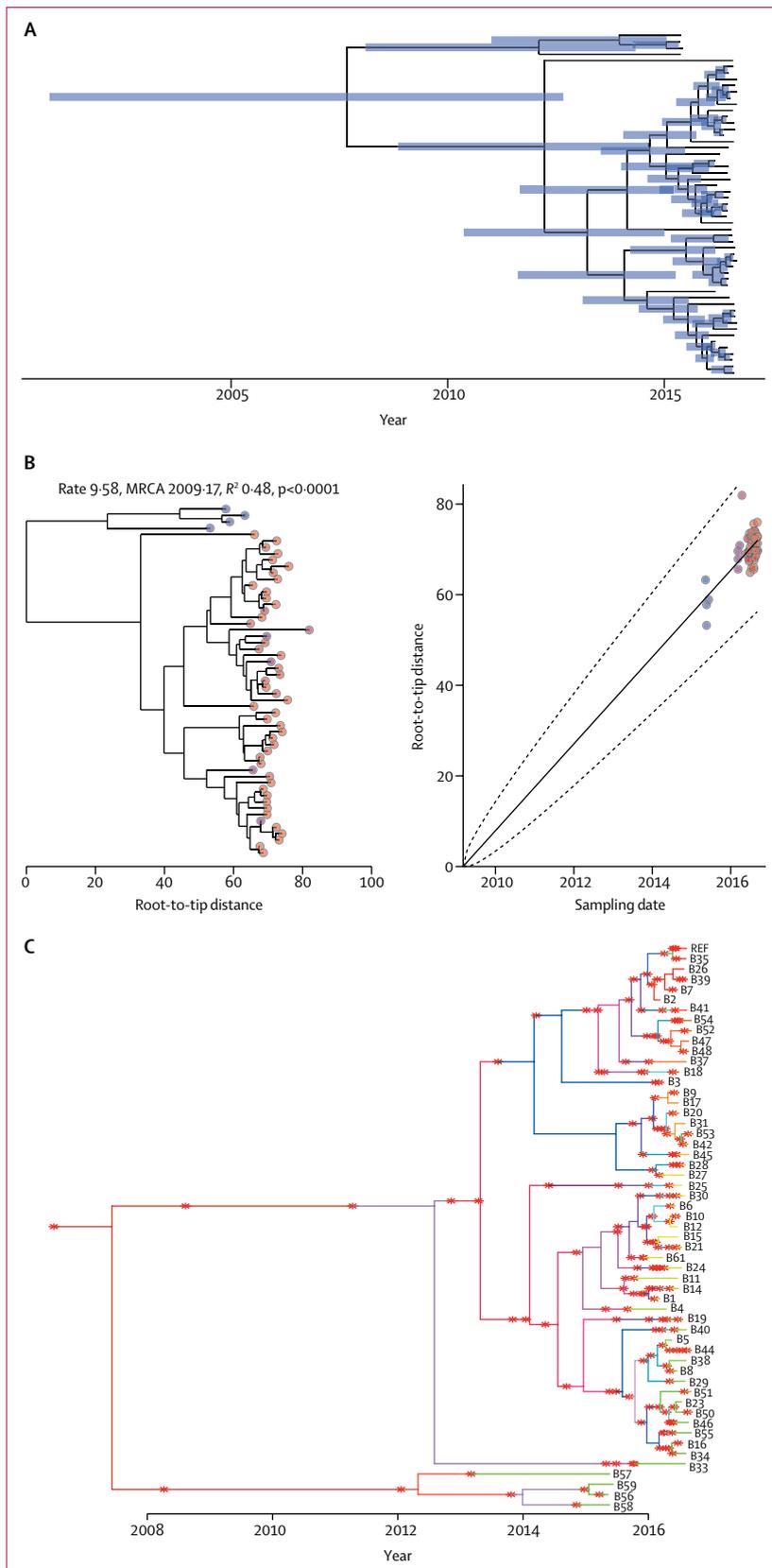
## Results

Between March 1, and Dec 31, 2016, 321 blood cultures were done on the EFSTH neonatal ward, of which 178 (55%) were positive with a clinically significant isolate (appendix p 6). 49 episodes of *B cepacia* bacteraemia from 47 newborns were reported in the neonatal ward between March 8 and Aug 29 (figure 1). The index *B cepacia* case, patient B1, was referred from a private clinic to the critical care ward of the neonatal unit in March (appendix p 7). This isolate (B1) was sensitive to recommended antibiotics. Patient B2 was admitted a day after patient B1, and patient B3 was admitted the day after patient B2; both patients B2 and B3 were referred from the same government hospital to the critical care ward, but both died after 24 h of admission. All three isolates (B1, B2, and B3) had the same antibiogram. An outbreak was not suspected at this time, but basic infection control was initiated and prolonged antibiotic use curbed (figure 1). With the identification of a fourth case (patient B4) in April, enhanced cleaning of cots and incubators was introduced. Between May 16 and July 27, 2016, 23 episodes of *B cepacia* bacteraemia (patients B5–B27) were reported in the critical care ward, each with similar antibiogram to previous isolates. Further infection control measures

	Mean pairwise SNP distance	Pairs
<b><i>Burkholderia</i></b>		
2016 only	2.18 (2.08–2.29)	1225
2015 only	7.33 (3.10–11.57)	6
2015 vs 2016	13.55 (13.27–13.82)	200
<b><i>Klebsiella</i></b>		
ST1535 ( <i>Klebsiella quasipneumoniae</i> subspecies <i>similipneumoniae</i> )	0.82 (0.70–0.93)	528
ST39 ( <i>Klebsiella pneumoniae</i> )	0.48 (0.24–0.71)	21
Data are n (95% CI) or n. SNP=single nucleotide polymorphism.		

**Table 1:** Summary of the genetic distances between isolates in the clusters computed as mean pairwise SNP distance

were introduced as a result. When 22 more cases (patients B28–B49) were identified in the critical care ward in August (isolates B32, B34, and B47 corresponding to patients 32, 34, and 47, were later identified as three separate episodes of infection in the same neonate), an outbreak was suspected and environmental samples were collected to rule out a reservoir (appendix p 8). Samples from randomly selected sponges used on the ward by mothers to bath their newborns, each grew multiple pathogens, including *K pneumoniae*, but not *B cepacia* (appendix p 9). *B cepacia* (isolates B50–B55) with identical antibiogram-to-clinical isolates were found in intravenous



fluid supplements and antibiotics (appendix p 10). On the neonatal ward, intravenous infusion mixtures were prescribed by doctors, mixed and administered by nurses in the ward areas, from a narrow selection of intravenous fluids and single-dose vials of parenteral electrolyte supplements. Multiple vials of antibiotics were simultaneously reconstituted or diluted with fluid drawn from a single bottle of intravenous fluid before being administered to the newborns; residual volumes of these reconstituted or diluted antibiotics were then pooled and reused. Procedural lapses observed included repeated insertion of a single syringe into an infusion bottle, storage of in-use injections and intravenous fluids on an open medicine trolley next to the sink, and poor hand hygiene. Of the 36 newborns with outcome data, 18 (50%) died on admission (appendix p 7). No further cases were detected between September and December.

We sequenced the *B cepacia* patient isolates B1–B49, as well as six environmental *B cepacia* isolates (B50–B55). We sequenced clinical isolates from six patients not thought to be associated with the outbreak: five historical isolates (B56–B60) from five newborns in the neonatal ward between May and August, 2015, and one isolate (B61) from a boy aged 4 years admitted to the general paediatric ward during the neonatal unit outbreak. Sequence reads from four patient isolates (B13, B22, B32, and B49), and one historical isolate (B60) were unavailable because of failed DNA extraction or sequencing library preparation. The historical isolates from 2015 formed a monophyletic clade, and the outbreak isolates from 2016 formed four subclades (I–IV) with short branches (figure 2). The initial three outbreak isolates from week 11 (B1, B2, and B3) clustered on separate branches on the phylogenetic tree. Subsequent isolates B4, B6, and B61 were closer to B1 than isolates B2 and B3. Isolate B5, an early isolate from week 21, was also placed on a separate subclade (IV), which encompassed three environmental isolates (B50, B51, and B55). Other environmental isolates were placed on subclades I (B53) and II (B52 and B54). Two isolates from a neonate (patient 32) with recurrent positive cultures were placed on subclades iv (B34) and II (B47). The average genetic distance between the outbreak isolates from 2016 of 2.18 SNPs (95% CI 2.08–2.29, range 0–9) was

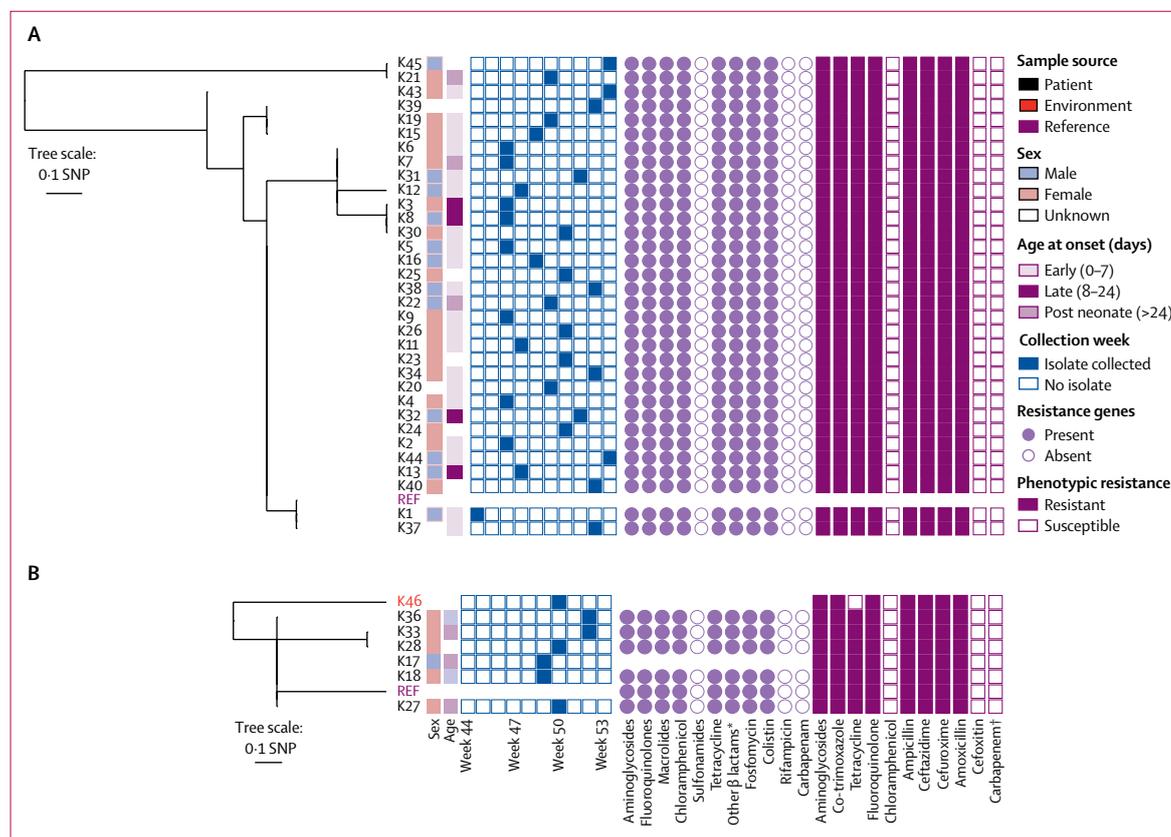
**Figure 3: Evolutionary timelines of outbreak *Burkholderia cepacia* isolates and the putative transmission routes that contributed to the evolution of the outbreak *B cepacia* strain**

(A) Time-dated phylogenetic tree with internal nodes annotated. Bars indicate the 95% highest posterior density intervals. (B) A root-to-tip analysis to estimate the time since the most recent common ancestor. The blue nodes indicate isolates from 2015, and the red nodes are isolates from 2016. (C) The time-dated phylogenetic tree annotated with transmission routes inferred using TransPhylo, shows the final iteration of 1000 Markov chain Monte Carlo iterations assuming a gamma distribution of generation times: branches are coloured to represent hosts (both sampled and inferred unsampled hosts). Changes in branch colour correspond to changes in host (ie, inferred transmission), and are marked with an asterisk. The timescale on the x-axis indicates the estimated timescale for the evolution of the strains. MRCA=most common recent ancestor.

significantly lower ( $p < 0.0001$ ) than the average genetic distance between isolates recovered in 2015 (7–33 SNPs, 3·10–11·57, 0–12; table 1). On average, the 2016 isolates differed from the 2015 isolates by 13·55 SNPs (13·27–13·82, 10–20). The historical isolates from 2015 provided a timescale to attempt a phylogenetic dating of this dataset using a Bayesian approach. A time-dated phylogeny was generated and a root-to-tip analysis was done to estimate the time since the most recent common ancestor. The dating suggests that the 2015 and 2016 isolates might have diverged from a common recent ancestor as early as 2009 (95% highest posterior density interval 2001–2013) and evolved separate lineages over time (figure 3). The dating suggests that each of the four subclades (I–IV) had a common recent ancestor around 2015 (figure 3). *B. cepacia* was probably an endemic contaminant in the neonatal ward, and there might have been at least four separate introductions in the ward and the hospital at large, which eventually gave rise to outbreaks. However, a single introduction of reservoir with a diverse inoculum cannot be ruled out. The time-dated phylogeny was used with Markov chain Monte Carlo methods to inform the potential

transmission patterns that shaped the outbreak. This analysis gave insights into the potential timescales for the evolution of the outbreak strains and highlighted the potential contribution of unsampled hosts in the transmission chain (figure 3).

The index *K. pneumoniae* case (K1) was a neonate aged 6 days admitted in October, 2016 (appendix p 11). The isolate from this index case (K1) was ESBL producing and resistant to gentamicin, ciprofloxacin, ceftazidime, cefuroxime, and cefepime, but sensitive to meropenem and imipenem. Within 11 days of the index case, three other newborns (patients K2, K3, and K4) acquired *K. pneumoniae* with the same antibiogram as patient K1, prompting fears of an outbreak. 41 additional patients (K5–K45) were identified between Nov 8, and the end of December. Although infection prevention and control measures introduced earlier were still in place, environmental sampling detected three *K. pneumoniae* isolates (K46, K47, and K48) from samples of intravenous fluids in use on the neonatal ward, all with the same antibiogram as those from the cases. No further cases were detected in January 2017.



**Figure 4:** The phylogeny of the *Klebsiella quasipneumoniae* subspecies *similipneumoniae* (A) and *Klebsiella pneumoniae* (B) outbreak isolates annotated with patient age, sex, a timeline of when isolates were collected, and antibiotic resistance patterns

Isolate generic identifications are shown and coloured by source: patient, environmental, or reference genome. Where applicable, sex is shown, and age is given as a categorical variable of early onset (0–7 days) or late onset (8–27 days). The presence of resistance genes conferring resistance to common classes of antibiotics are given alongside the phenotypic antibiotic resistance patterns. SNP=single nucleotide polymorphism. \*Cephalosporins and carbapenems (excluding imipenem and meropenem). †Imipenem and meropenem only.

	ST39	ST1535
Aminoglycosides	Resistant: <i>aac(3)II</i> , <i>aac(6)Ib</i> , <i>aadA</i> , <i>aph(3'')Ia</i> , <i>aph(3'')Ib</i> , <i>aph(6)-Id</i> , <i>strA</i> , <i>strB</i>	Resistant: <i>aac(3)IIa</i> , <i>aac(6)Ib</i> , <i>aph(3'')Ia</i> , <i>aph(6)-Id</i> , <i>strA</i> , <i>strB</i>
β-lactam (excluding carbapenems)	Resistant: <i>bla<sub>CTX-M-15</sub></i> , <i>bla<sub>OXA-5'</sub></i> , <i>bla<sub>TEM-30'</sub></i> , <i>bla<sub>AMPH</sub></i> , <i>bla<sub>SHV-187'</sub></i> , <i>bla<sub>7</sub></i>	Resistant: <i>bla<sub>CTX-M-15'</sub></i> , <i>bla<sub>OXA-5'</sub></i> , <i>bla<sub>TEM-30'</sub></i> , <i>bla<sub>AMPH</sub></i> , <i>bla<sub>OXP-B-5</sub></i>
Trimethoprim	Resistant: <i>dfrA14</i> , <i>dfrA12</i>	Resistant: <i>dfrA14</i>
Tetracycline	Resistant: <i>tetD</i>	Resistant: <i>tetA</i>
Quinolones or fluoroquinolones	Resistant: <i>qnrB</i>	Resistant: <i>qnrB</i>
Fosfomycin	Sensitive	Sensitive
Sulfonamide	Resistant: <i>sul1</i> , <i>sul2</i>	Resistant: <i>sul2</i>
Macrolide	Resistant: <i>mphA</i>	Sensitive
Chloramphenicol	Resistant: <i>catB4</i>	Resistant: <i>catB4</i>
Yersiniabactin lineage	..	<i>ybt 15</i> ; <i>ICEKp11</i>

\*The known resistance genotypes shown were present in at least 90% of isolates belonging to each respective genotype.

**Table 2: Summary of the predicted genotypic resistance patterns and yersiniabactin alleles in the outbreak *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* subspecies *similipneumoniae* sequence types**

The genomic analysis of the *K pneumoniae* outbreak included isolates from 39 patients and one environmental sample (K46). The genome data identified contemporaneous *Klebsiella* outbreaks due to two related but distinct species—sequence type 1535 (ST1535) *Klebsiella quasipneumoniae* subspecies *similipneumoniae* (n=33, capsule locus type KL114) and ST39 *K pneumoniae* (n=7, capsule locus type KL23). Each genotype consisted of closely related variants of the same strain: ST1535 isolates differed from one another by mean 0.82 core genome SNPs (95% CI 0.70–0.93, range 0–5) and the ST39 isolates differed from one another by mean 0.48 SNPs (0.24–0.71, 0–1; table 1; figure 4). The low sequence diversity suggests each of these clusters has a very recent common ancestor, consistent with the short timeframe; however, precise dating was not possible because of the low diversity.

Among the *Klebsiella* isolates, we observed appreciable concordance between the multidrug phenotypic resistance pattern and the genotypic resistance, except for chloramphenicol, to which strains were classed as susceptible despite the presence of the *catB4* resistance allele. Both STs harboured multiple *bla* genes conferring resistance to β-lactams, including that encoding for the ESBL CTX-M-15, which was conserved in all strains (table 2). The combination of virulence genes differed between the two strain clusters. The acquired siderophore yersiniabactin (allele *ybt 15*, *ICEKp11*) was present in ST1535 strains, but absent in ST39. The colibactin genotoxin and other acquired siderophores were absent from both outbreak strains.

## Discussion

We report sequential hospital-acquired outbreaks of *B cepacia* and multidrug-resistant, ESBL-producing *Klebsiella* bacteraemia in The Gambia's only newborn unit over a 10-month period. WGS and a time-dated phylogeny

suggest that *B cepacia* has been endemic in the neonatal ward, potentially for several years. Similar sustained endemicity has been described in other countries,<sup>29</sup> and might be linked to the low mutation rate of *Burkholderia* spp.<sup>30</sup> Genomic analysis also revealed existence of two contemporaneous outbreaks due to *K pneumoniae* (ST39) and a related species, *K quasipneumoniae* subsp *similipneumoniae* (ST1535). The highly conserved nature of both species suggests a more recent introduction into the hospital environment than *B cepacia*. *K quasipneumoniae* is often misidentified as *K pneumoniae* by routine clinical microbiological diagnostics and the two species are difficult to distinguish without genome data.<sup>31</sup> An outbreak of New Delhi metallo-β-lactamase (NDM-5) containing *K quasipneumoniae* subsp *similipneumoniae* neonatal bacteremia with different ST (ST476) was reported in Nigeria in 2019.<sup>11</sup> Both ST1535 and ST39 were resistant to β-lactam antibiotics, excluding carbapenems, because of ESBL expression, and resistant to other important classes of antibiotics such as aminoglycosides and fluoroquinolones. Unlike the Nigerian NDM-5 variant, which showed elevated carbapenem resistance, *K quasipneumoniae* subsp *similipneumoniae* isolates identified during our outbreak were sensitive to carbapenems. To the best of our knowledge, this is the second report of the virulence factor yersiniabactin (*ICEKp*) in *K quasipneumoniae* subsp *similipneumoniae* (a single ST477 KL15 carrying *ICEKp2* was reported in Singapore in 2019<sup>32</sup>). Our limited clinical outcome data demonstrate high case fatalities among newborns in both outbreaks. The high fatality of infants infected with *B cepacia* despite treatment and susceptible antibiograms supports existing evidence that there is poor agreement between in vitro testing for *B cepacia* antibiotic susceptibility patterns and in-vivo response.

The observation that most patients received intravenous fluids and bacteria with identical or nearly identical genotypes were isolated from in-use parenteral preparations implicate contamination of intravenous fluids. Unopened fluids and antibiotic solutions were determined to be sterile, suggesting the contamination occurred extrinsically because of procedural lapses. Similar extrinsic contamination of intravenous fluids has been described in other sub-Saharan African countries.<sup>33</sup> Intrinsic contamination of medicinal products during manufacture can also lead to outbreaks.<sup>34</sup> Although intravenous fluids and antibiotics form part of the standard care provided to most newborns admitted in our unit, and are an epidemiological link between most of the outbreak cases, other routes of acquisition could not be ruled out. The *K pneumoniae* outbreak coincided with the peak admission period (September to December) in the neonatal ward, during which cot occupancy increases 100-fold.<sup>12</sup> High rates of neonatal gastrointestinal colonisation with ESBL-producing *K pneumoniae* and high stool bacterial loads provide a reservoir for spread from baby to baby via the hands of mothers and staff,<sup>35</sup> and some of the cases might have

occurred because of cross-contamination from intestinally colonised babies.

Outbreaks of *B cepacia* are strongly associated with contaminated hospital tap water, when *B cepacia* is dislodged from the biofilm of distribution lines and tank surfaces and released into the water supply on increased water demand,<sup>36</sup> whereas *K pneumoniae* outbreaks have been associated with severe water shortages.<sup>37</sup> Despite having sinks in the neonatal ward, there was no running water when both outbreaks occurred—water collected from the central hospital tank was drawn from a single bucket and used for handwashing and bathing of patients. Given the proximity of the medicine trolley to the ward sink and poor hand hygiene, we hypothesise that the hospital water might have been the source of infection, although none of the water samples were positive for any of the outbreak organisms because of the common difficulties in pathogen recovery resulting from inadequate sampling or variations in pathogen load within the water system.<sup>38</sup> Interestingly, during investigation of the *B cepacia* outbreak, we isolated *K pneumoniae* from a random selection of sponges used to bath babies (appendix p 9), but these isolates were not stored and therefore could not be sequenced for comparison with later outbreak isolates. It is thus possible that both pathogens were introduced through different sources and were able to proliferate and spread because of neonatal vulnerability and lapses in infection control.

Although the outbreaks reported in this study were eventually brought under control by a review and change in hand hygiene and infection prevention and control measures on the neonatal ward, they drew attention to three important issues. First was the multiple use of single-dose antibiotics and insufficiency of commercially manufactured volume-appropriate premixed intravenous fluids for neonatal use in countries of low and middle income. The small volumes of dextrose and electrolyte solutions necessitate mixing and handling of these fluids from several multidose vials during preparation, thereby increasing the probability of extrinsic contamination. Second was the absence of policies, standards, and protocols to support decision-making or the prevention and control of infection. Studies in Zambia<sup>39</sup> and in Senegal<sup>40</sup> have demonstrated that a low-technology, hospital-acquired infection control bundle can be implemented in a hospital neonatal unit with limited resources. In addition to educating staff and mothers on hand hygiene, assignment of cleaning responsibilities and antibiotic stewardship, bundles should include proper standards for handling parenteral fluids, including written procedures for admixture of intravenous fluids, a central pharmacy area for manipulation of fluids and drugs, and laminar flow hoods for management of parenteral fluids.<sup>41,42</sup> Last was the need to improve the quality of infection prevention and control procedures, particularly environmental cleaning. The potential for

contaminated environmental surfaces to facilitate nosocomial infection depends on several factors, including frequency with which organisms contaminate environmental surfaces, ability of pathogens to remain viable on surfaces, location of pathogen reservoirs, hand-touch frequency of surfaces, adequate level of contamination required to pose a transmission risk, and pathogen infectivity index.<sup>43</sup> We assumed that implementation of hand washing, fumigation, disinfection, and cleaning would lead to a sudden halt in the occurrence of both outbreaks, but this was not the case. Unlike *Klebsiella* spp, which can survive for months on dry surfaces, *B cepacia* does not survive on dry surfaces for more than 1 week, but can survive for many months in water or respiratory droplets on environmental surfaces.<sup>44</sup> Visual assessment is insufficient for defining cleanliness; equally important are the cleaning agent, frequency of cleaning, methods, equipment, monitoring, and implementation of standards for surface cleanliness.<sup>45</sup>

Our study has some limitations. Many newborns were referred for hospitalisation who did not have blood cultures done, and we might have missed some episodes of bacteraemia. Our inference might have been limited by the fact that we only picked and sequenced single colonies, which precludes our ability to account for within-host diversity and multistrain infection. Although the outbreak investigation identified the likely transmission route of the reported hospital-acquired infections, the original sources remain unclear. This absence of the original source is important, given that the *Burkholderia* outbreak apparently ended before the outbreak investigation occurred, and the *Klebsiella* outbreak occurred despite the infection control measures. Nevertheless, this is the first reported use of WGS to support an infection-control investigation in sub-Saharan Africa. Using WGS to the infection-control investigation allowed us to classify the *B cepacia* and *Klebsiella* infections as nosocomial outbreaks and not community-acquired infections, and enabled more precise identification of bacterial species and transmission pathways than with standard microbiological techniques. In the future, the required sequencing technologies might become more rapidly available also in low-income and middle-income countries, with the aim to rapidly identify transmission dynamics and inform control efforts. Our study has important implications for preventing newborn deaths from hospital-acquired infections in countries of low and middle income, where even the supportive treatments administered might be leading to infection transmission.

#### Contributors

UO, SD, and BK conceptualised the study. UO wrote the first draft of the Article, prepared figure 1 and the clinical data tables. SD led the pathogen isolation and antimicrobial sensitivity testing. SMAZ, MJH, and DN coordinated the outbreak investigation. KLD did the DNA extraction. SDB and EB sequenced the isolates and conceptualised the bioinformatics analysis. MS, EB, and SDB did the bioinformatic analysis and prepared the phylogenetic trees. KEH provided guidance on the interpretation of genomic analysis. NJH provided guidance on

interpretation of microbiological data. JEL and BK provided guidance on interpretation of clinical and epidemiological data. All authors provided input to the overall direction and content of the Article, and provided input to, saw, and approved the final version.

#### Declaration of interests

BK reports grants from maternal and neonatal health research, including vaccine studies, outside the submitted work. KLD reports grants from UK Research and Innovation and European and Developing Countries Clinical Trials Partnership, outside the submitted work. All other authors declare no competing interests.

#### Data sharing

The genome data has been deposited in the European Nucleotide Archive (appendix pp 12–13).

#### Acknowledgments

This work is supported by the Medical Research Council (MRC) Unit The Gambia at the London School of Hygiene & Tropical Medicine (LSHTM). Work at the MRC Unit is jointly funded by the UK MRC and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the European and Developing Countries Clinical Trials Partnership programme supported by the EU. UO was supported by an MRC PhD Fellowship. BK is supported by funding from the MRC (MC\_UP\_A900/1122; MC\_UP\_A900/115; MR/K007602/1) and acknowledges the contribution of a Thrasher Senior Investigator award to this work. NJH was supported by a Research Fellowship by the German Research Foundation (HO 6280/1–1). We thank Muhammed Afolabi for providing technical support at the Edward Francis Small Teaching Hospital; Baderinwa Abatan, Helen Brotherton, and Abdou Gai for assistance with clinical data collection; Ousman Secka and Bolarinde Lawal for oversight of environmental sampling; Buntung Ceeseay and Mamadou Jallow for microbiological support; and Jonas Lexow for providing technical support at the MRC Unit The Gambia at LSHTM.

#### References

- United Nations Inter-agency Group for Child Mortality Estimation. Levels & trends in child mortality: report 2019. Estimates developed by the UN inter-agency group for child mortality estimation, 2019. <https://www.unicef.org/reports/levels-and-trends-child-mortality-report-2019> (accessed Jan 15, 2020).
- Liu L, Oza S, Hogan D, 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* 2016; **388**: 3027–35.
- Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. *Lancet* 2005; **365**: 1175–88.
- Okomo U, Akpalu ENK, Le Doare K, et al. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. *Lancet Infect Dis* 2019; **19**: 1219–34.
- Harris SR, Cartwright EJ, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis* 2013; **13**: 130–36.
- Köser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N Engl J Med* 2012; **366**: 2267–75.
- Durand G, Javerliat F, Bes M, et al. Routine whole-genome sequencing for outbreak investigations of *Staphylococcus aureus* in a national reference center. *Front Microbiol* 2018; **9**: 511.
- Polonsky JA, Baidjoe A, Kamvar ZN, et al. Outbreak analytics: a developing data science for informing the response to emerging pathogens. *Philos Trans R Soc Lond B Biol Sci* 2019; **374**: 20180276.
- Azarian T, Maraqa NF, Cook RL, et al. Genomic epidemiology of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *PLoS One* 2016; **11**: e0164397.
- Harris SR, Cartwright EJ, Török ME, et al. Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis* 2013; **13**: 130–36.
- Brinkac LM, White R, D'Souza R, Nguyen K, Obaro SK, Fouts DE. Emergence of New Delhi metallo- $\beta$ -lactamase (NDM-5) in *Klebsiella quasipneumoniae* from neonates in a Nigerian hospital. *MSphere* 2019; **4**: e00685-18.
- Okomo UA, Dibbasey T, Kassama K, et al. Neonatal admissions, quality of care and outcome: 4 years of inpatient audit data from The Gambia's teaching hospital. *Paediatr Int Child Health* 2015; **35**: 252–64.
- Gladstone RA, Lo SW, Lees JA, et al. International genomic definition of pneumococcal lineages, to contextualise disease, antibiotic resistance and vaccine impact. *EBioMedicine* 2019; **43**: 338–46.
- Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012; **19**: 455–77.
- Harris SR, Feil EJ, Holden MT, et al. Evolution of MRSA during hospital transmission and intercontinental spread. *Science* 2010; **327**: 469–74.
- Page AJ, Taylor B, Delaney AJ, et al. *SNP-sites*: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom* 2016; **2**: e000056.
- Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014; **30**: 1312–13.
- Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol Biol Evol* 2016; **33**: 1870–74.
- Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* 2016; **44**: W242–5.
- Didelot X, Croucher NJ, Bentley SD, Harris SR, Wilson DJ. Bayesian inference of ancestral dates on bacterial phylogenetic trees. *Nucleic Acids Res* 2018; **46**: e134.
- Didelot X, Fraser C, Gardy J, Colijn C. Genomic infectious disease epidemiology in partially sampled and ongoing outbreaks. *Mol Biol Evol* 2017; **34**: 997–1007.
- Hunt M, Mather AE, Sánchez-Busó L, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microb Genom* 2017; **3**: e000131.
- Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012; **67**: 2640–44.
- Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895–903.
- Chen L, Yang J, Yu J, et al. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res* 2005; **33**: D325–28.
- Lam MMC, Wick RR, Wyres KL, et al. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in *Klebsiella pneumoniae* populations. *Microb Genom* 2018; **4**: e000196.
- Lam MMC, Wyres KL, Judd LM, et al. Tracking key virulence loci encoding aerobactin and salmochelin siderophore synthesis in *Klebsiella pneumoniae*. *Genome Med* 2018; **10**: 77.
- 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**: e00197-18.
- Jacobson M, Wray R, Kovach D, Henry D, Speert D, Matlow A. Sustained endemicity of *Burkholderia cepacia* complex in a pediatric institution, associated with contaminated ultrasound gel. *Infect Control Hosp Epidemiol* 2006; **27**: 362–66.
- Dillon MM, Sung W, Lynch M, Cooper VS. The rate and molecular spectrum of spontaneous mutations in the gc-rich multichromosome genome of *Burkholderia cenocepacia*. *Genetics* 2015; **200**: 935–46.
- Long SW, Linson SE, Ojeda Saavedra M, et al. Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*. *MSphere* 2017; **2**: e00290-17.
- Octavia S, Kalisvar M, Venkatachalam I, et al. *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* define the population structure of blaKPC-2Klebsiella: a 5 year retrospective genomic study in Singapore. *J Antimicrob Chemother* 2019; **74**: 3205–10.

For the genome data see <https://www.ebi.ac.uk/ena/browser/view/PRJEB20799>

- 33 Moodley P, Coovadia YM, Sturm AW. Intravenous glucose preparation as the source of an outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* infections in the neonatal unit of a regional hospital in KwaZulu-Natal. *S Afr Med J* 2005; **95**: 861–64.
- 34 Song JE, Kwak YG, Um TH, et al. Outbreak of *Burkholderia cepacia* pseudobacteraemia caused by intrinsically contaminated commercial 0.5% chlorhexidine solution in neonatal intensive care units. *J Hosp Infect* 2018; **98**: 295–99.
- 35 Desta K, Woldeamanuel Y, Azazh A, et al. High gastrointestinal colonization rate with extended-spectrum  $\beta$ -lactamase-producing *Enterobacteriaceae* in hospitalized patients: emergence of carbapenemase-producing *K. pneumoniae* in Ethiopia. *PLoS One* 2016; **11**: e0161685.
- 36 Lucero CA, Cohen AL, Trevino I, et al. Outbreak of *Burkholderia cepacia* complex among ventilated pediatric patients linked to hospital sinks. *Am J Infect Control* 2011; **39**: 775–78.
- 37 Akindele JA, Gbadegesin RA. Outbreak of neonatal *Klebsiella* septicaemia at the University College Hospital, Ibadan, Nigeria. Appraisal of predisposing factors and preventive measures. *Trop Geogr Med* 1994; **46**: 151–53.
- 38 Anaissie EJ, Penzak SR, Dignani MC. The hospital water supply as a source of nosocomial infections: a plea for action. *Arch Intern Med* 2002; **162**: 1483–92.
- 39 Mwananyanda L, Pierre C, Mwansa J, et al. Preventing bloodstream infections and death in Zambian neonates: impact of a low-cost infection control bundle. *Clin Infect Dis* 2019; **69**: 1360–67.
- 40 Landre-Peigne C, Ka AS, Peigne V, Bougere J, Seye MN, Imbert P. Efficacy of an infection control programme in reducing nosocomial bloodstream infections in a Senegalese neonatal unit. *J Hosp Infect* 2011; **79**: 161–65.
- 41 Calil R, Marba ST, von Nowakonski A, Tresoldi AT. Reduction in colonization and nosocomial infection by multiresistant bacteria in a neonatal unit after institution of educational measures and restriction in the use of cephalosporins. *Am J Infect Control* 2001; **29**: 133–38.
- 42 Hernández-Ramos I, Gaitán-Meza J, García-Gaitán E, León-Ramírez AR, Justiniani-Cedeño N, Avila-Figueroa C. Extrinsic contamination of intravenous infusates administered to hospitalized children in Mexico. *Pediatr Infect Dis J* 2000; **19**: 888–90.
- 43 Mitchell BG, Wilson F, Dancer SJ, McGregor A. Methods to evaluate environmental cleanliness in healthcare facilities. *Healthc Infect* 2013; **18**: 23–30.
- 44 Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 2006; **6**: 130.
- 45 Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. *J Hosp Infect* 2009; **73**: 378–85.