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# What caused the outbreak of ESBL-producing *Klebsiella pneumoniae* in a neonatal intensive care unit, Germany 2009 to 2012? Reconstructing transmission with epidemiological analysis and whole-genome sequencing

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## ABSTRACT

**Objective:** We aimed to retrospectively reconstruct the timing of transmission events and pathways in order to understand why extensive preventive measures and investigations were not sufficient to prevent new cases.

**Methods:** We extracted available information from patient charts to describe cases and to compare them to the normal population of the ward. We conducted a cohort study to identify risk factors for pathogen acquisition. We sequenced the available isolates to determine the phylogenetic relatedness of *Klebsiella pneumoniae* isolates on the basis of their genome sequences.

**Results:** The investigation comprises 37 cases and the 10 cases with ESBL (extended-spectrum beta-lactamase)-producing *K. pneumoniae* bloodstream infection. Descriptive epidemiology indicated that a continuous transmission from person to person was most likely. Results from the cohort study showed that 'frequent manipulation' (a proxy for increased exposure to medical procedures) was significantly associated with being a case (RR 1.44, 95% CI 1.02 to 2.19). Genome sequences revealed that all 48 bacterial isolates available for sequencing from 31 cases were closely related (maximum genetic distance, 12 single nucleotide polymorphisms). Based on our calculation of evolutionary rate and sequence diversity, we estimate that the outbreak strain was endemic since 2008.

**Conclusions:** Epidemiological and phylogenetic analyses consistently indicated that there were additional, undiscovered cases prior to the onset of microbiological screening and that the spread of the pathogen remained undetected over several years, driven predominantly by person-to-person transmission. Whole-genome sequencing provided valuable information on the onset, course and size of the outbreak, and on possible ways of transmission.

## Strengths and limitations of this study

- We highlight the complexity of nosocomial outbreaks and outbreak investigations.
- We illustrate how microbiological and epidemiological methods may uncover pieces of a puzzle that only when taken together can resolve the whole picture of an outbreak situation.
- Our study is limited to data and isolates collected during the outbreak investigation. We could not retrieve epidemiological data on all neonates and had to make use of the available isolates collected during the investigation.

## INTRODUCTION

On 28 February 2012, a German neonatal intensive care unit (NICU) was closed due to a large outbreak of ESBL-producing *Klebsiella pneumonia*. It has remained closed ever since (as on February 2015).[1] In total, 37 infants were affected by this outbreak. Among these, 10 cases developed a bloodstream infection.

Local public health authorities were first informed of the outbreak in September 2011 and convened an infection control team. The hospital conducted a press conference on 2 November 2011 to inform the public about the outbreak. From then the outbreak was discussed in the media for months, and a parliamentary commission of the Bremen parliament was established.[1 2] By the time the media was informed, the index case was suspected to be a neonate who tested positive for the outbreak strain in July 2011.

Throughout the outbreak, control measures were established with emphasis on

hand hygiene, contact precautions, cohorting, personalisation of all medication and care products, and limiting access to the units (eg, patient visits were restricted to parents). Admission of new infants in the NICU was ceased and the ward was closed on 5 November 2011 for renovation; all movable material was discarded. The NICU reopened on 9 January 2012; however, five new cases were detected in the NICU in February.

On 25 October 2011, a microbiological screening of all infants in the NICU was introduced and then extended to a twice weekly screening of all patients in the paediatric clinic and an admission screening. More than 270 staff members were screened twice, in November 2011 and February 2012. Mothers on the maternal ward were screened starting on 5 November 2011 (perianal swabs). Mothers and staff were all screened negative for the outbreak strain. More than 650 environmental samples were collected from the NICU, other paediatric wards, the maternity ward and the delivery room (>350 in 2011 and >300 in 2012), including air, water, disinfectants and medical products. Only five environmental samples were ESBL-*K. pneumoniae* positive: a diaper scale, a sharps disposal, a baby soother (used by a case) in 2011, a baby soother (used by another case) and a cardboard box of gloves (9 days after final closure of the ward) in 2012.

Outbreaks due to multidrug-resistant *K. pneumoniae* have been reported particularly in NICUs.[3–5] They may be difficult to control as *K. pneumoniae* can colonise the gastrointestinal tract of patients without causing signs of infections,[6] acting as reservoirs for continued transmission.[7 8] Other German hospitals have faced *K. pneumoniae* outbreaks, but we are not aware of a nosocomial outbreak in Germany that drew comparable public attention and that has led to similar drastic consequences.

## OBJECTIVE

We investigated this large, complex outbreak by combining epidemiological analysis with whole-genome sequencing. A combination of epidemiological analysis and high-resolution, whole-genome sequencing has been shown to be valuable for investigating outbreaks with various bacterial pathogens, including *K. pneumoniae*.[9–12] In particular, we aimed to retrospectively reconstruct the timing of transmission events, date the start of the outbreak, and further elucidate possible sources in order to understand why extensive measures and investigations were insufficient to prevent new cases.[1]

## METHODS

### Setting

The paediatric clinic in Bremen, Germany, with about 160 beds, has more than 11 000 inpatients and about 40 000 outpatients per year, including paediatric surgery. Neonates were treated in the NICU, the paediatric intensive care unit (PICU), a paediatric surgical ward and a rooming-in unit.

### Confirmed case

Any patient treated in the paediatric clinic in Bremen with the ESBL-*K. pneumoniae* outbreak strain (DNA macrorestriction) detected prior to 15 May 2012.

### Probable case

Patients having received treatment in the paediatric clinic between 1 January 2011 and 15 May 2012, with ESBL-*K. pneumoniae* record, but with no isolate available for bacterial typing.

### Data source

We extracted information from patient charts, the hospital information system and the laboratory information system, and performed a retrospective case search within these data sources. Variables such as gestational age, birth weight, sex, exposures (eg, alimentation, procedures), outcomes (eg, microbiological findings), as well as information on the mother and the delivery of all cases belonging to the outbreak and all non-cases in the cohort study were extracted.

*Descriptive epidemiology* was applied to all cases for whom information was retrieved. Subsequently, we compared cases born and identified between 1 January and 31 October 2011 to all neonates born during the same time span, who received treatment in the paediatric clinic for more than 12 h within the first 7 days of life (standard data set for quality assessment).

*A retrospective cohort study* included infants who were in the NICU between 9 February and 28 February 2012 to analyse reoccurrence of the outbreak strain. The time of exposure for cases was defined as time since birth or last negative screening until 1 day before first detection of ESBL-*K. pneumoniae*. The time of exposure for non-cases was the length of hospital stay within the study period. We tested the association of single exposures with being a case, and tested combined variables as invasive measures and those exposures that led to frequent manipulation.

### Statistical analyses

We calculated relative risks and p values (significance level of $p<0.05$) using Poisson regression in Stata (Stata V.12: StataCorp LP; 2011).

### Bacterial strain typing

All conserved ESBL-*K. pneumoniae* that had been isolated were analysed using DNA macrorestriction with *XbaI* and subsequent pulsed-field gel electrophoresis (PFGE) was performed as previously described[13] (see online supplementary methods).

### Genome sequencing

We generated a reference genome sequence for the outbreak strain from isolate *K. pneumoniae* 234/12 (Pacific Biosciences, 454/Roche, and Illumina technologies; see online supplementary methods). For annotation, we

used the RAST web pipeline (http://rast.nmpdr.org/)[14] and the PHAST tools.[15]

Resequencing of DNA from 46 *K. pneumoniae* isolates was performed by applying Illumina technology (see online supplementary methods). We mapped the resulting paired-end reads to the reference genome sequence to identify sequence variation (see online supplementary methods). We inferred multilocus sequence types (STs) from genome sequences. Sequence data were submitted to the Sequence Read Archive at NCBI (BioProject PRJNA 235888; http://www.ncbi.nlm.nih.gov/sra).

### Phylogenetic analyses

An alignment of core genome single nucleotide polymorphisms (SNPs) assisted in the reconstruction of the isolates' phylogeny using the PhyML module in Seaview, V.4.2.3. We then estimated evolutionary rates and divergence dates from an alignment of genome sequence dates with the isolates' recovery dates, by using BEAST V.1.7.4 (http://beast.bio.ed.ac.uk; see online supplementary methods).

### Ethics

A formal ethical review process and approval was not required for this outbreak investigation in accordance with article 25, section 1 of the German Infection Protection Act of 2001.

## RESULTS

### Descriptive epidemiological analysis

In total, we identified 37 cases (31 (84%) confirmed cases and 6 (16%) probable cases (epidemiological data were retrievable for 35 (95%) cases)). Using retrospective case search we identified 6 cases (2 confirmed and 4 probable) that were tested ESBL-*K. pneumoniae* positive prior to the supposed index case (case 12, July 2011). There were 46 isolates available for sequencing: 38 isolates from 31 cases, 5 environmental isolates and 2 isolates from patients from the hospital without epidemiological link and 1 isolate of a similar strain from Poland.

The majority (n=33, 89%) of cases was born preterm (gestational age, <37+0), 10 (27%) cases developed a bloodstream infection with the outbreak strain, and 7 (19%) died (all-cause mortality).

Between 30 April 2011 and 28 February 2012, at least one case was permanently hospitalised in the paediatric clinic; 65% of cases were on the same ward as another case within 7 days prior to first detection, and 90% of cases had been treated in the NICU (figure 1).

We analysed a data set of neonates born between January and November 2011 and treated in the paediatric clinic (n=310, including 20 cases). The cases had a lower birth weight and a lower gestational age, and developed sepsis significantly more often than non-cases (table 1). However, when adjusted for birth weight and

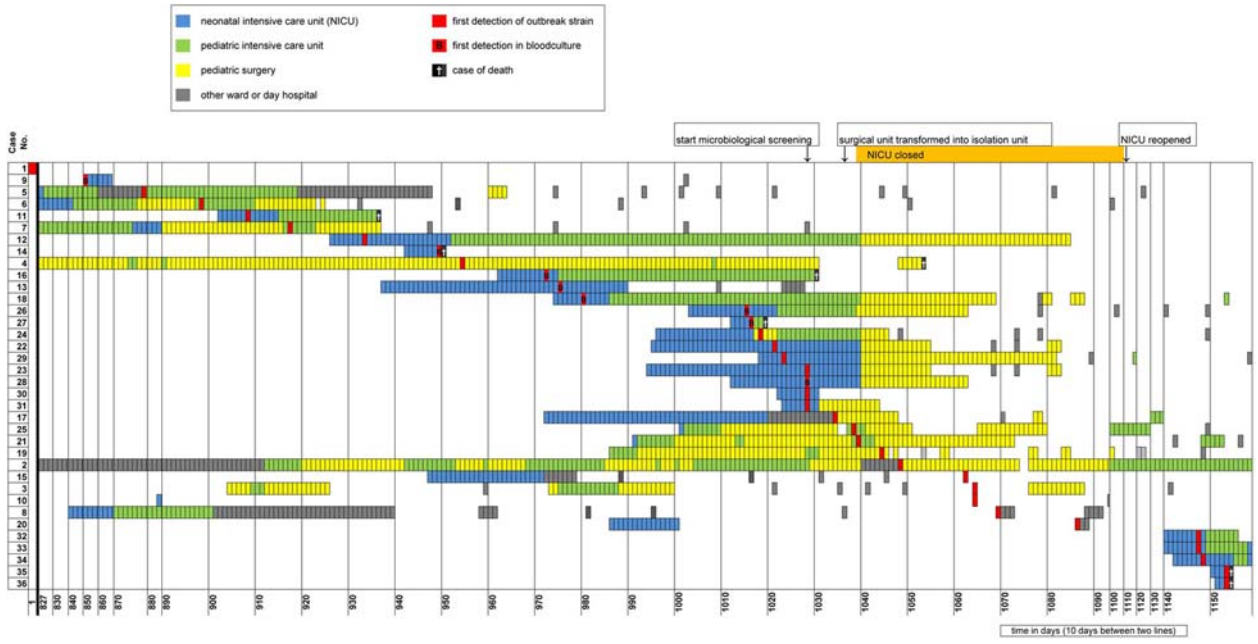


**Figure 1** Timeline of the outbreak. The index patient (case 1), isolate from ≈01.01.2009 (day 1 of the outbreak) was retrospectively found to belong to the outbreak. Eight hundred and fifty days later the second case was tested positive. Black line between day 1 and day 827 represents time span with no additional information on cases. Time intervals of 10 days between vertical lines compressed whenever additional information was not lost. Data on case 37 are not presented in this figure. (Note: After discharge many infants came back to hospital for day hospital visits during which they were treated by members of the same team treating infants on neonatal intensive care unit (NICU) and paediatric intensive care unit. Sixty-five per cent of cases were on the same ward as another case within 7 days prior to first detection. Thirty-three cases were exposed to the NICU.)

Table 1 Comparison of cases with non-cases

| | | Cases (n=20) | Non-cases (n=290) | RR* | 95% CI | p Value |
|---|---|---|---|---|---|---|
| Sex | Male | 13 (65%) | 149 (51%) | 1.69 | 0.63 to 5.02 | 0.36 |
| Multiples | Yes | 8 (40%) | 76 (26%) | 1.79 | 0.64 to 4.77 | 0.30 |
| Birth weight (g) | Median (range) | 1105 (620–2640) | 2400 (468–4,87) | 0.26 | 0.14 to 0.49 | <0.001 |
| Gestational age (weeks) | Median (range) | 27+5 (24+2–36+2) | 35+6 (24+3–42+6) | 0.77 | 0.695 to 0.858 | <0.001 |
| Fatalities† | Yes | 4 (20%) | 22 (8%) | 2.73 | 0.66 to 8.46 | 0.16 |
| Sepsis | Yes/no | 9 (45%) | 41 (14%) | 4.25 | 1.56 to 11.29 | 0.005 |

Twenty cases born and identified between 1 January and 31 October 2011, and 290 non-cases born in the same time period treated in the paediatric clinic, univariable analysis.
*Relative risk (RR) and p values calculated using Poisson regression or exact Poisson regression (where possible) and corresponding 95% CI.
†All fatalities among cases and non-cases counted, not differentiating causes.

gestational age, the association with sepsis was no longer significant (RR 1.50; 95% CI 0.56 to 4.02).

## Cohort study

Twenty infants were included in this cohort, comprising 5 cases and 15 non-cases. One case had a *Klebsiella* sepsis and two cases died. Birth weight, gestational age and gender did not differ significantly between cases and controls. Analysing exposures we found a significant association with the combined variables as proxy for 'frequent manipulation' (RR 1.44; 95% CI 1.02 to 2.19 for 1 point increase). Infants had up to 10 points in the combined variable, indicating an increased risk up to 14-fold of becoming a case (table 2).

## Genetic population structure

ST514 had not been observed in Germany previously, but was reported in two neonatal patients at a university hospital in Poland in 1996.[16] De novo genome sequencing of our isolate 234/12 by combining Pacific Biosciences and Illumina sequencing technologies revealed that the outbreak strain displayed large-scale genomic differences to the 'historic' ST514 isolate (316/12) from 1996, including the acquisition and/or restructuring of prophages. We then resequenced the genomes from 46 *K. pneumoniae* isolates by applying Illumina sequencing technology (see online supplementary table S1), and polymorphisms in the core genome were identified by mapping sequencing reads to the genome sequence from the reference isolate 234/12. All 44 ST514 outbreak isolates carried the ESBL gene $bla_{\text{CTX-M-15}}$ and the β-lactamase genes $bla_{\text{TEM-1}}$ and $bla_{\text{SHV-63}}$. All these isolates were resistant to ampicillin, cefotaxime and ceftazidime, but they were susceptible to carbapenems (see online supplementary table S1).

Among ST514 isolates from the outbreak in the paediatric clinic, we discovered a total of only 61 SNPs, illustrating their close relationship. Two short runs of adjacent SNPs (each consisting of 2 SNPs with a distance on the chromosome of 84 and 358 base pairs, respectively) and one homoplasious SNP (see online supplementary table S2) were signatures of potential recombination events. For phylogenetic analyses, we removed these five potentially recombined SNPs from the data set to avoid any obfuscation of the phylogenetic signal. Of the remaining 56 SNPs, 13 were parsimony-informative (ie, they occurred in >1 haplotype), enabling the reconstruction of a unique phylogenetic tree (homoplasy index, 0.0; figure 2). When this tree was rooted with the genome from an older ST514 isolate (316/12, Poland, 1996), the earliest isolate from the paediatric clinic (267/12, from 2009) sat closest to the root (see online supplementary figure S1). In a minimum spanning tree for our data set

Table 2 Cohort study 2012; selected devices and procedures during the study period (9 February to 28 February 2012)

| Personal characteristics, exposure | | Cases (n=5) | Non-cases (n=15) | RR | CI | p Value |
|---|---|---|---|---|---|---|
| Sex | Male | 3 (60%) | 7 (47%) | 1.5 | 0.2 to 18.0 | 1.00 |
| Multiples | Yes/no | 0 (0%) | 2 (13%) | 1.3 | 0.0 to 9.82 | 1.00 |
| Birth weight (g) | Median (range) | 1800 (498–3000) | 2300 (613–4210) | 1.0 | 1.0 to 1.0 | 0.50 |
| Gestational age (weeks+days) | Median (range) | 31+2 (24+2–36+3) | 34+2 (24+1–42+1) | 0.9 | 0.7 to 1.1 | 0.21 |
| Central venous catheter | Yes | 4 (80%) | 6 (40%) | 4.0 | 0.4 to 197.0 | 0.38 |
| Umbilical catheter | Yes | 4 (80%) | 2 (13%) | 9.3 | 0.9 to 459.6 | 0.06 |
| Arterial catheter | Yes | 3 (60%) | 0 (0%) | 8.5 | 1.0 to 101.8 | 0.05 |
| Mechanical ventilation | Yes | 3 (60%) | 4 (27%) | 2.8 | 0.3 to 33.4 | 0.47 |
| Surfactant | Yes | 3 (60%) | 2 (13%) | 4.5 | 0.5 to 53.9 | 0.21 |
| 'Frequent manipulation' (0–10)* | Median (range) | 7 (4–10) | 4 (0–7) | 1.4 | 1.0 to 2.2 | 0.04 |

Univariable exact Poisson regression and corresponding 95% CI.
*Combined variables as proxy for 'frequent manipulation' (administration of eye drops (1)+continuous positive airway pressure (1)+radiograph (1)+ECG (1)+physiotherapy (1)+umbilical catheter (1)+central venous catheter (1)+arterial catheter (1)+urinary catheter (1)+administration of surfactant (1)+mechanical ventilation (1)+blood transfusion (1)).

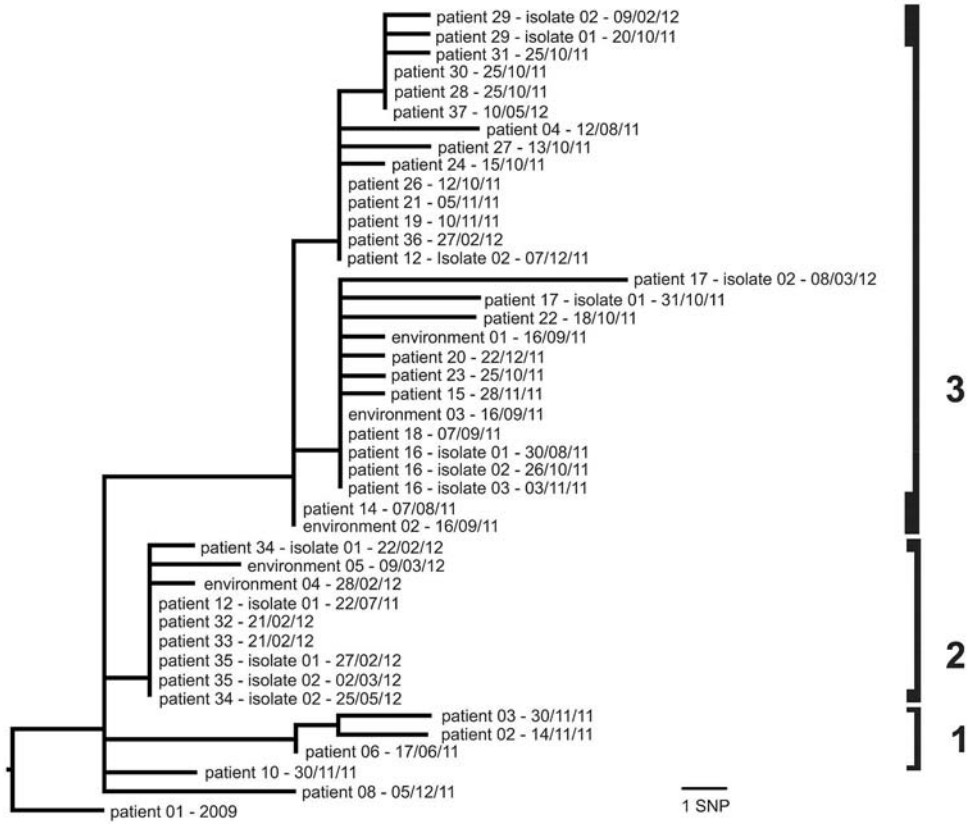


**Figure 2** Maximum-likelihood phylogenetic tree based on sequence variation in the core genome (5.2 Mio base pairs) from ESBL-producing *Klebsiella pneumoniae* isolates representing the outbreak investigated. The tree was rooted by using isolate 316/12 (ST514, collected in Poland in 1996). Patient numbers, environmental sample numbers and isolation dates are indicated. Where multiple isolates from individual patients were available, these are numbered consecutively. Phylogenetic clades 1, 2 and 3 are indicated. SNP, single nucleotide polymorphism; ST, sequence type.

of 43 isolates, 40 isolates clustered in three clades (clades 1, 2 and 3; figure 3) that were fully congruent with clades in a maximum-likelihood tree (figure 2). However, the minimum spanning tree allowed ancestral isolates to occupy internal nodes, which may reflect the underlying transmission tree more closely.[17] While DNA macrorestriction (PFGE) enabled identification of the outbreak strain, pulsotype subgroups differing from each other by one or two bands were not concordant with SNP-based phylogeny, since several of these subgroups were found in more than one of the three phylogenetic clades (figure 2).

### Evolutionary rate and age of outbreak strain

Based on our set of genome sequences from isolates that had been sampled serially over time, we estimated that this *K. pneumoniae* strain has accumulated point mutations in its core genome at a rate of three mutations per genome and year ($5.5\times10^{-7}$ substitutions per nucleotide site and year; 95% CI $2.9\times10^{-7}$ to $8.4\times10^{-7}$). This short-term evolutionary rate is similar to rates recently reported for *Clostridium difficile*,[18] *Mycobacterium tuberculosis*[19] and *Vibrio cholerae*,[20] but lower than that of *Staphylococcus aureus*.[21 22] Based on this evolutionary rate and the sequence diversity of our data set, the time estimation of the most recent common ancestor of the *K. pneumoniae* outbreak strain was the beginning of 2008 (95% CI 2006 to 2009). Of note, virtually the same rate and dates were calculated after removal of the single isolate from 2009.

### Diversity of *K. pneumoniae* within individual patients

From each of six individual patients, two to three isolates were available. In two of these cases (patients 16 and 35), two to three isolates collected up to 65 days apart had fully identical genomes, and in two cases (patients 34 and 29), the isolates sampled 93 and 112 days apart, respectively, differed by one to two SNPs (figure 2). This result is consistent with the average short-term evolutionary rate and suggests that the diversity within individual patients may be low. In contrast, two isolates from patient 17 collected 127 days apart differed by nine-point mutations, yet clustered together in the phylogenetic tree (figure 2). Two isolates from patient 12 sampled 138 days apart were affiliated with separate clusters in the phylogenetic tree (figure 2). These findings could be due to reinfection with a related strain and/or the continuous presence of diverse pathogen populations within these individuals.

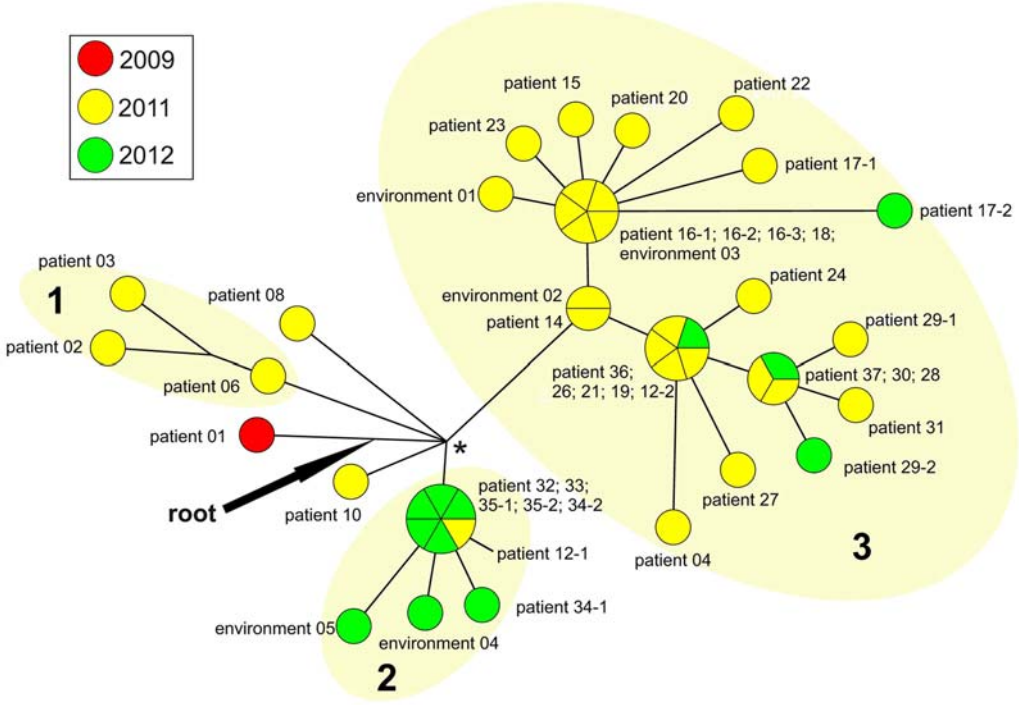


**Figure 3** Minimum spanning tree (constructed by using Bionumerics software) based on the same sequence data as the maximum-likelihood tree in figure 2. The root of the tree was determined by comparison to the genome from isolate 316/12 (ST514, collected in Poland in 1996). Colours indicate isolation dates. Where multiple isolates from individual patients were available, these are numbered consecutively. Phylogenetic clades 1, 2 and 3 are indicated. The asterisk marks the multifurcation point in the tree that is referred to in the text (ST, sequence type).

## DISCUSSION

Epidemiological links and microbiological results suggest an outbreak of 37 affected infants (31 confirmed cases). ST514 has not been reported from other sites in Germany, including hospitals, which indicates that this ST is rare. We provide evidence for a person-to-person transmission and for undetected cases, which helps to better understand why the extensive hygiene measures may have been insufficient to stop transmission.

### Extent of the outbreak

Our investigation indicates that undetected cases must have occurred within the paediatric clinic prior to the onset of microbiological screening. Before screening was introduced, cases were identified only if they had developed signs of infection or if mechanical ventilation had prompted a microbiological screening (it was current practice on the ward to perform microbiological screening of sputum in mechanically ventilated infants). Accordingly, the proportion of cases with *Klebsiella* sepsis was 50% before implementation of screening, decreasing to 10% after. When microbiological screening started in the NICU (25 October 2011), seven new cases were detected within 12 days, also suggesting a formerly high prevalence of undetected cases. One case, a term-born infant who had stayed in the NICU in June 2011, in whom the colonisation with the outbreak strain was only detected in December 2011, after the infant had been actively screened for ESBL-*K. pneumoniae* months after discharge. No other risk factors had been reported for this case, leaving the stay in the NICU the most plausible time of outbreak strain acquisition (figure 1).

Accordingly, outbreak isolates sampled in 2011 displayed considerable genomic diversity, since all three clades (figure 3) were already present, and the multifurcation point in the minimum spanning tree (labelled with an asterisk in figure 3) reflects the lack of specimens from before 2011.

Before the introduction of microbiological screening, the timeframe during which transmission could occur was often long (up to 445 days). Extensive barrier precautions were not in place during time periods in which carriers of the outbreak strain remained undetected. Risk factors for being a case in our investigation were low gestational age and low birth weight, which confirms risk factors identified in previous outbreak reports.[11]

### Beginning of the outbreak

The spread of the pathogen remained undetected for approximately 3 years. Prior to our retrospective case search, the outbreak was assigned a start date of 22 July 2011. However, we were able to identify earlier cases, including one from 2009: Based on genome sequence variation that we found among outbreak isolates and considering the evolutionary rate at which point

mutations accumulated over time, we estimated that the outbreak strain had been endemic in the hospital since 2008.

### Pathogen reservoir and transmission pathways

Person-to-person pathogen spread is most likely, although we could not directly identify a responsible vehicle of transmission. The outbreak strain was continuously present in the NICU during the study period (figure 1). Genome sequence data supported patient-to-patient transmission as the principle route of pathogen spread, since in the minimum spanning tree (figure 3) all but one furcation nodes in clades 1, 2 and 3 are occupied by isolates sampled from patients, rather than from parents, staff or any environmental source. In contrast, repeated introductions of the pathogen from a common source would have resulted in a star-like tree, and introductions from multiple external sources would have resulted in greater diversity that would have been only partially represented by the strain collection from the ward.

Evidence of the critical role of hand hygiene was provided by the cohort study, which showed a significant association with the compound score for 'frequent manipulation' (table 2). When environmental investigations were intensified, increased numbers of pathogens (not the outbreak strain) were detected on personnel hands, gowns and hand contact places (ie, pager or box of gloves).

Understaffing and overcrowding have repeatedly been described as risk factors for increased infection rates and the occurrence of outbreaks.[23] Nonetheless, the median patient/nurse ratio in the NICU in 2011 was 3.0, and repeatedly one nurse had to take care of more than five neonates (data not shown).

Drastic measures were implemented to control the outbreak during the last months of 2011, including stopping admissions, closure and renovation of the NICU. Nevertheless, phylogenetic clades 2 and 3 were present before and after reopening of the NICU (figures 2 and 3). Moreover, the positions of isolates from 2012 in the minimum spanning tree show that at least two different genotypes (cases 32–36, clades 2 and 3; figure 3) were introduced into the ward following the renovation.

Repeated screenings of staff did not detect any ESBL-producing *K. pneumoniae*. Likewise, the outbreak strain was neither detected among mothers on the maternal ward nor among patients from other wards, or from other hospitals within the area. It is unlikely that the outbreak clone was endemic in the wider geographic area. Genomic relatedness of *K. pneumoniae* isolates supported the notion that patients constituted a reservoir for the pathogen, as several neonates were colonised with unique strains for several months (figure 2). Notably, closely related isolates were collected from each of two patients (cases 17 and 29) before and after renovation of the NICU (figure 2), suggesting that the pathogen may have been reintroduced into the NICU by person-to-person transmission. Case 29 was rehospitalised in the PICU 20 days before birth of case 32. Case 17 was rehospitalised in the PICU only 1 day before birth of case 32. Further undetected cases hospitalised in 2012 may have reintroduced the outbreak strain through outpatient visits in 2012.

### Environmental investigation

Environmental screenings yielded five *K. pneumoniae* isolates that were closely related to the outbreak strain (see online supplementary table S1). Coincidentally, genomes from these isolates (sampled from a box of gloves, a diaper scale, a sharps disposal and baby soothers, respectively) occupied peripheral positions of the minimum spanning tree (figure 2), ruling out these contaminated materials as spreading hubs. The finding of the outbreak strain in a box of gloves 9 days after closure of the ward demonstrates the remarkable viability of *Klebsiella* on inanimate surfaces. Additional boxes of gloves (including unopened ones) tested negative, suggesting that the single positive tested box of gloves was most likely contaminated by insufficiently disinfected hands. Tests performed by a specialised laboratory showed that the outbreak strain was sensitive to the disinfectants used in the clinic.

## LIMITATIONS

Most data on exposures were collected from patient records. Within these we found systematic inconsistencies in documentation and missing data on epidemiologically important information, like room number, attending personnel and incubator number. Use of skin disinfectant (eg, for umbilical cord care) was more frequently documented in known cases than in non-cases, even though a standard procedure applied to all newborns. Furthermore, by extending barrier precautions and moving the NICU, medical procedures and thereby, the exposures may have changed.

While genome sequencing provided improved discriminatory power when compared with other typing methods,[9 11 12] the short-term evolutionary rate in *K. pneumoniae* of three base substitution per genome per year on average, as measured here, is unlikely to allow the reliable tracking of specific transmission events between individuals, which occur on much shorter time scales. Resolution could have likely been improved by including multiple isolates from each individual neonate, which were not sufficiently available in the present investigation.

## CONCLUSIONS AND RECOMMENDATIONS

Late detection and thereby, delayed extensive response may have contributed to the difficulties in controlling this outbreak. Our analyses suggest that there were numerous unknown cases and that measures, such as cohorting and barrier nursing, were only applied to known cases. The outbreak spread from the NICU to other wards of the paediatric clinic, and readmissions of

known and unknown cases may have reintroduced the outbreak strain into the NICU.

While in the present analysis, extensive sequencing was applied retrospectively only, genomic diversity of *K. pneumoniae* isolates from patients and environmental samples clearly indicated that the duration and extent of the outbreak had been underestimated. Further, sequence data provided evidence for the patients being the reservoir of the pathogen and it ruled out environmental contamination as a major route of spread.

Surveillance systems for outbreak detection with standardised evaluation of pathogen clusters should be established, especially on wards with highly susceptible populations. Having this valuable information during real-time outbreak situations would improve infection control and prevention measures by elucidating the start and extent of the outbreak, confirming possible transmission routes and identifying most probable sources.

Since January 2012, the German Commission for Hospital Hygiene and Infectious Disease Prevention (KRINKO) recommends weekly microbiological screening for multiresistant Gram-negative pathogens and Methicillin-resistant *Staphylococcus aureus* (MRSA) in all very low birthweight infants (<1500 g) in Germany.[24 25]

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**Contributors** SH, JH, WW, EV, MAS and TE were part of the outbreak team and conducted the epidemiological outbreak investigations. MK, MS, AR, PWD, AN, YP, GW and UN conducted the microbiological investigations. SH, CE, TE and UN conceptualised the studies. SH, CE, TE and UN drafted the manuscript. All authors critically revised the manuscript and approved the final version. SH is corresponding author and guarantor.

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