Investigation of an outbreak caused by antibiotic-susceptible *Klebsiella oxytoca* in a neonatal intensive care unit in Norway

Torunn Gresdal Rønning (torunn.gresdal.ronning@stolav.no)[1], Christina Gabrielsen Aas[1,2], Ragnhild Støen[2,3], Kåre Bergh[1,2], Jan Egil Afset[1,2], Mari Sagli Holte[4], Andreas Radtke[2,4]

1.Department of Medical Microbiology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway
2.Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway
3.Department of Neonatal Intensive Care, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway
4.Unit of Infection Control, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Keywords
Infection control measures, *Klebsiella oxytoca*, Neonatal intensive care unit, Outbreak, Whole-genome sequencing

Correspondence
Torunn Gresdal Rønning, St. Olavs Hospital, Laboratoriesenteret, Erling Skjalgssons gate 1, Trondheim, Norway.
Tel: +47 93838678 |
Email: torunn.gresdal.ronning@stolav.no

Received
7 September 2018; accepted 17 September 2018.

DOI:10.1111/apa.14584

ABSTRACT

Aim: *Klebsiella* spp. have been stated to be the most frequent cause of neonatal intensive care unit (NICU) outbreaks. We report an outbreak of *Klebsiella oxytoca* in a NICU at a tertiary care hospital in Norway between April 2016 and April 2017. This study describes the outbreak, infection control measures undertaken and the molecular methods developed.

Methods: The outbreak prompted detailed epidemiological and microbial investigations, where whole-genome sequencing (WGS) was particularly useful for both genotyping and development of two new *K. oxytoca*-specific real-time PCR assays. Routine screening of patients, as well as sampling from numerous environmental sites, was performed during the outbreak. A bundle of infection control measures was instigated to control the outbreak, among them strict cohort isolation.

Results: Five neonates had symptomatic infection, and 17 were found to be asymptomatically colonised. Infections varied in severity from conjunctivitis to a fatal case of pneumonia. A source of the outbreak could not be determined.

Conclusion: This report describes *K. oxytoca* as a significant pathogen in a NICU outbreak setting and highlights the importance of developing appropriate microbiological screening methods and implementing strict infection control measures to control the outbreak in a setting where the source could not be identified.

INTRODUCTION

Klebsiella oxytoca is a Gram-negative rod which can be found in a wide range of environments, and is capable of colonising both the skin and the intestinal tract of humans (1). This bacterial species can also cause a variety of nosocomial infections in humans ranging from mild to severe disease (2). Outbreaks in neonatal intensive care units (NICUs), including fatalities, have also been described (3–5). The colonisation rate of *K. oxytoca* in a healthy adult population has been reported in the range 1.6–9% (6,7), but less is known about colonisation in neonates. *Klebsiella* spp. have been stated to be the most frequent cause of NICU outbreaks (8). This is, however, best documented for antibiotic-resistant strains (9,10). Known risk factors for outbreaks in NICUs include patient crowding and understaffing, low gestational age and birthweight of the patients, long stay in the NICU and indwelling catheters (11,12).

An increased incidence of invasive *K. oxytoca* infection (two cases of sepsis and one peritonitis) in the NICU during the period April–June 2016 initially raised awareness of a possible clustering of *K. oxytoca* infections in the NICU in our hospital. Two of three *K. oxytoca* isolates displayed an identical genotypic profile by pulsed-field gel electrophoresis

Abbreviations
BW, Birthweight; ECMO, Extracorporeal membrane oxygenation; GA, Gestational age; NICU, Neonatal intensive care unit; PFGE, Pulsed-field gel electrophoresis; SCAI, Simmons citrate agar with 1% inositol; WGS, Whole-genome sequencing.

Key notes

- We report an outbreak of *Klebsiella oxytoca* in a neonatal intensive care unit at a tertiary care hospital in Norway between April 2016 and April 2017.
- Five neonates had symptomatic infection, and 17 were found to be asymptomatically colonised.
- The development of appropriate microbiological molecular screening methods and implementing strict infection control measures were important to control the outbreak.

(PFGE). The notion of a possible outbreak was substantiated as two additional *K. oxytoca* isolates with an identical PFGE profile were found in a nasopharyngeal aspirate of a neonate and in breastmilk from a mother of another neonate at the NICU during July–August 2016. Together, these findings prompted instigation of a series of infection control measures, epidemiological and microbial investigations, screening of patients and sampling of numerous environmental sites.

We herein report the spread of a virulent, antibiotic-susceptible *K. oxytoca* strain in a NICU and describe the development of microbiological methods, including development of outbreak-specific screening tests, extensive microbiological testing of patients and environmental sites, and infection control measures which were undertaken to control the outbreak.

MATERIALS AND METHODS

Setting

The NICU at St. Olavs hospital, Trondheim University Hospital, is a 21-bed level III NICU with approximately 500 annual admissions. Treatment is provided to all categories of preterm infants and includes all levels of intensive care except extracorporeal membrane oxygenation (ECMO). The unit also admits neonates requiring surgery and has shared responsibility for all neonatal surgery (except open heart surgery) in Norway together with Oslo University Hospital. The NICU consists of four bays (rooms), each with a capacity of up to five infants. There are two isolates and a step-down unit comprising seven single-family rooms. Parents are allowed entrance to the unit 24/7, but only those in the step-down unit can stay overnight. Adult beds are allowed beside the incubators, and parents are encouraged to have skin-to-skin contact with their baby (kangaroo care).

Screening strategy

Screening of all patients admitted to the NICU for faecal carriage of *K. oxytoca* started late September 2016, and four screenings of all admitted patients were performed during autumn 2016 (56 samples from 46 unique patients). From January to April 2017, weekly screenings were performed, and from April to September 2017, screenings were performed twice a week. The total number of screening samples until September 2017 was 748 (216 unique patients). To check whether the outbreak could be due to transmission from colonised women in the maternity ward, rectal samples from a number of women (n = 25) admitted for seven days or longer were also screened for faecal carriage. Some of the women were tested two or three times, due to long hospital stays. Breastmilk samples from mothers (n = 21) of babies admitted to the NICU were also analysed for *K. oxytoca*. Breastmilk samples from five of the mothers were collected and tested on multiple occasions because their child was admitted over an extended period. Employees were not screened for faecal carriage of *K. oxytoca*.

Environmental investigations

In an attempt to identify a possible environmental source of the outbreak, environmental sampling was performed. All sinks, showerheads and taps in the NICU were sampled, as well as all incubators and breastmilk pumps. Furthermore, samples collected in the NICU included handles of hand disinfection and soap dispensers, filters of the washer disinfector, computer keyboards and mice, air sampling from the ventilation system, soap from soap dispensers, bottles used for routine cleaning and disinfection, the keyboard, probe and gel of the ultrasound device, laryngoscopes, stethoscopes and a number of environmental surfaces. Nutritional solutions such as oral caffeine citrate and oral sucrose solution, infant formula and human milk fortifier were cultured. Outside the NICU, environmental samples from breastmilk pumps and sinks from the maternity wards and from the sink and an incubator (moisturiser) used to transport newborns from the operating room to the NICU were collected. In total, 86 environmental sites were sampled.

Infection control measures

A bundle of infection control measures was already in place in late July 2016 as a consequence of a *Salmonella* infection in a neonate (Table 1, Fig. 1).

After the detection of extensive spread of *K. oxytoca* among admitted neonates in January/February 2017, additional infection control measures (Table 1, Fig. 1) were

Table 1 Infection control measures used during the outbreak

First bundle of infection control measures (July/August 2016)
Outbreak control team appointed
Augmented cleaning and disinfection procedures initiated
Reinforcement of daily cleaning of every room
Review of responsibilities regarding cleaning of benches and medical instruments
Daily Virkon® disinfection of sinks
Disinfection of patient rooms after discharge
Regular meetings with cleaning personnel and hospital staff to ensure compliance with implemented measures
Replacement of breast pumps and some refrigerators
Revision and review of routines for handling of breastmilk
Revision and review of routines for handling of milk formula and fortifiers
Strict temperature control and regular cleaning of refrigerators used for storage of breastmilk, formulae and nutrition
Closing of parent kitchen inside the NICU
Second bundle of infection control measures (February 2017)
Increased focus on indications and management of central lines, and reduction of indwelling time
Personnel trained in WHO hand hygiene observation tool* and observations of colleagues
Limitations of two patients/incubators per room
Cohort isolation of patients (2/3 wards)
Nursing staff assigned exclusively to one ward on each shift
Pasteurisation of all human donor milk

NICU = Neonatal intensive care unit.
*Ref. (24).

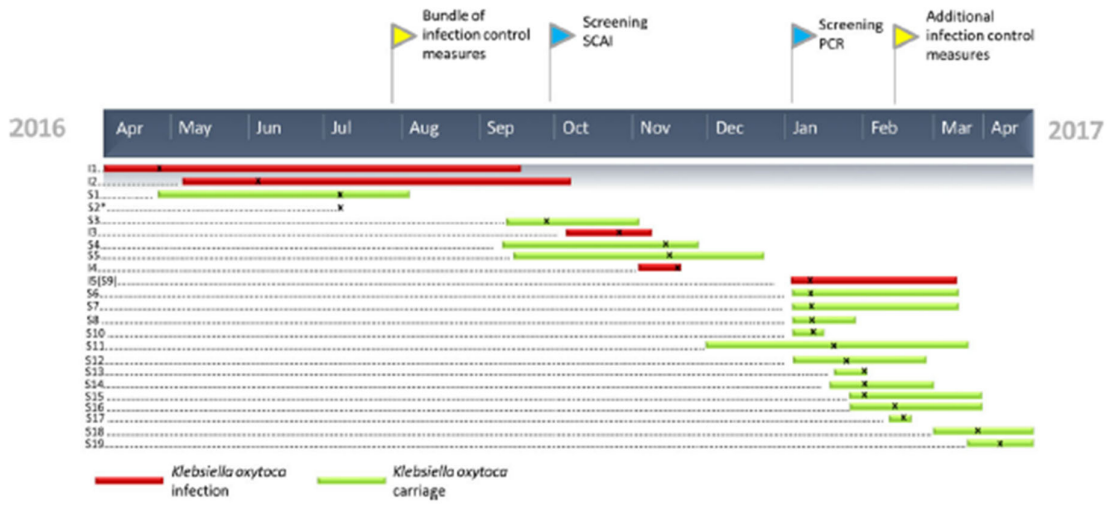

Figure 1 Timeline illustrating the *Klebsiella oxytoca* outbreak at the neonatal intensive care unit (NICU) at St. Olavs hospital, Trondheim University Hospital. Each case is displayed as a box, with *K. oxytoca* carriage and infection indicated by green and red colour, respectively, and x indicating the time of first detection. S2* is the *K. oxytoca* outbreak strain found in breastmilk. The position and length of boxes are chronological and correspond to the length of stay in the NICU.

enforced. In order to minimise crowding in the larger rooms, the capacity per room was limited to two patients. Cohort isolation of colonised neonates with dedicated staff was furthermore initiated: patients with faecal carriage of *K. oxytoca* were placed in one ward (ward 1) and recently admitted patients with no proven faecal carriage were placed in a separate ward (ward 2). A new case of *K. oxytoca* in ward 2 five weeks later prompted the establishment of a third cohort (ward 3) at a different location in the hospital, where patients with the lowest gestational age (GA) (<28 weeks) and/or patients with central lines were admitted.

Microbiological investigations

Sample collection

Faecal specimens were collected with FecalSwab™ (Copan Italy, Brescia, Italy) from diapers of neonates. Nasopharyngeal aspirate was collected and transported in virus transport medium without antibiotics. Blood and peritoneal fluid were collected in BD BACTEC™ Peds Plus™/F Culture vials and incubated in a Bactec FX blood culture system (Becton Dickinson, Franklin Lakes, NJ, USA). Environmental samples were collected with moist ESwab™ for bacterial culture. Air sampling from the ventilation system was performed using airIDEAL 3P airsampler (bioMérieux, Marcy-l'Étoile, France).

Screening and antibiotic susceptibility

Simmons citrate agar with 1% inositol (SCAI) (13) was used for screening of *K. oxytoca* in faecal and environmental samples from late September 2016. Environmental samples were additionally cultured on blood agar. MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) was used for identification of bacterial isolates. Antibiotic susceptibility of strains was tested using the disc diffusion method and interpreted according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

Species- and strain-specific real-time PCR

DNA for PCR was extracted from faecal specimens by NucliSENS easyMAG total nucleic acid extractor (bioMérieux). A *K. oxytoca*-specific real-time PCR method targeting the *pehX* gene (Table 2) was developed and used

Table 2 Primer and probe sequences for real-time PCR detection of *Klebsiella oxytoca* (*pehX*) and presumptive detection of the outbreak strain (*infB* 36)

Target	Primer/probe	Nucleotide sequence (5'-3')	Position in *K. oxytoca* strain CAV1335
pehX gene	Forward primer	ACT TCG GCT AAG CAC AGC AT	2033181–2033200
	Reverse primer	ARC TGA CGT TGG AAA AAC GC	2033301–2033282
	TaqMan probe	ATT TCG GCT ACC GTC ATG CCG TCA AT	2033230–2033205
infB 36	Forward primer	GGT ATG ATC ACC TTC CTA	4240061–4240078
	Reverse primer	GAT ATC CGT CGC CTG A	4240141–4240126
	TaqMan probe	CGT TCA CCT CTA TGC GTG CT	4240098–4240117

for all screening analyses on primary samples performed in 2017. All PCR-positive samples were cultured in an attempt to isolate *K. oxytoca* in pure culture for genotyping.

An allele-specific primer extension real-time PCR for use on primary samples targeting an outbreak-strain-specific SNP in the *infB* gene was developed for subsequent use in the follow-up period (Table 2). The ultimate nucleotide (A at position 18) of the forward primer represents a SNP in the *infB* 36 allele. The *infB* 36 allele-specific PCR was considered negative when the cycle threshold (CT) difference of the specimen examined was ≥6 compared to the *pehX* PCR result of the same specimen. In practice, the *infB* 36 allele-specific PCR was negative when the *K. oxytoca* strain tested was dissimilar to the outbreak strain.

Thermal cycling conditions for both PCR assays were as follows: activation of uracil-N-glycosylase (UNG) at 45°C for five minutes and initial denaturation at 95°C for three minutes, followed by 40 cycles of 95°C for five seconds, and 55°C for 30 seconds. The PCR mix used was PerfeCTa MultiPlex qPCR SuperMix (Quanta Biosciences, Gaithersburg, MD, USA).

Genotyping

Genotyping of *K. oxytoca* isolates was initially performed using PFGE as described by Bannermann et al. (14) with minor modifications. Similarity was interpreted using the criteria of Tenover (15). Briefly, cultures were grown overnight in Todd Hewitt broth and *Xba*1 (New England Biolabs, Beverly, MA, USA) was used for cutting. The electrophoresis was performed with a 10-hour first ramping phase (five to 15 seconds) and a 13-hour second phase (15–60 seconds). DNA isolation of *K. oxytoca* isolates prior to whole-genome sequencing (WGS) was performed on a M48 BioRobot and EZ1 Advanced XL Automated Nucleic Acid Purification System (Qiagen, Hilden, Germany). WGS was performed on a MiSeq system, using the Nextera XT DNA sample preparation kit and MiSeq® Reagent Kit v3 (600 cycle) (Illumina, San Diego, CA, USA). Assembly was performed using SPAdes Genome Assembler (16), annotation was done using Prokka (17), and core genome phylogeny was done using Roary (18) and FastTree (19,20). FigTree (21) was used for visualisation. Similarity was interpreted based on phylogenetic clustering and distances (transitions and transversions) between core genomes based on a 2 533 743-nucleotide core alignment and calculated using MEGA (22).

Case definition

A confirmed case was defined as a person who was colonised or infected with *K. oxytoca* that, based on

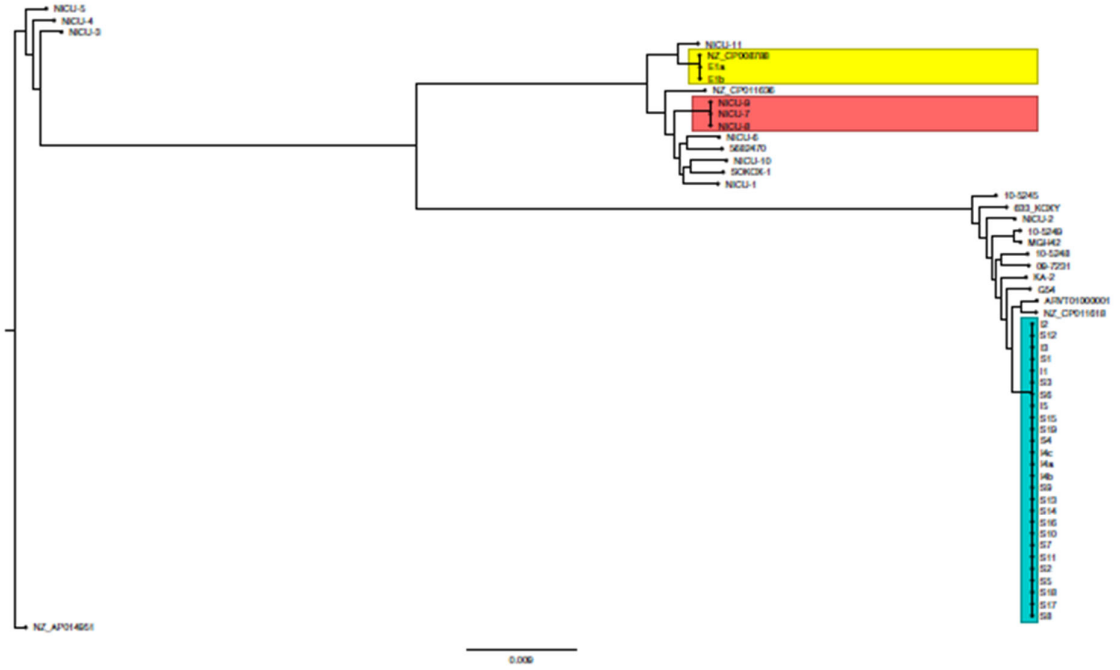

Figure 2 Core genome phylogeny based on whole-genome sequencing of *Klebsiella oxytoca* strains and selected *K. oxytoca* reference genomes. Strains that met the case definition are highlighted in blue. Environmental isolates from the neonatal intensive care unit (NICU) are highlighted in yellow. Strains marked in red are *K. oxytoca* isolated from faeces from late April 2017/early May 2017 (also considered as transmission of *K. oxytoca* in the NICU, but without any clinical manifestation). Scale represents substitutions per site.

genotyping with PFGE or WGS analysis, belonged to the same cluster as the index strain. The study was considered and approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (Project 2017/966).

RESULTS

Description of the outbreak

Within a one-year period (April 2016–April 2017), five neonates had symptomatic *K. oxytoca* infections, of which four suffered invasive infection (Fig. 1). Three of these patients had sepsis and/or pneumonia (I1, I5, and I4, respectively), and one died (I4). *Klebsiella oxytoca* was also isolated from peritoneal fluid in a neonate with perforated necrotising enterocolitis (I2), while another patient had conjunctivitis (I3). By screening of faeces, carriage of the outbreak strain was furthermore detected in 16 neonates, including one patient (S9/I5) who later developed sepsis. The outbreak strain was also found in one nasopharyngeal aspirate (S1) and one breastmilk specimen (S2).

Demographic characteristics

A total of 22 neonates admitted to the NICU were colonised or had infection with *K. oxytoca* during the outbreak. The median birthweight (BW) of these neonates was 1150 g (445–3355 g), and the median gestational age (GA) was 29 weeks (24–36). The length of stay varied from seven days to 197 days (median 39 days). The three patients with invasive *K. oxytoca* infection had indwelling central venous catheters at the onset of symptoms or within 24 hours prior to symptoms. Two of the patients were also intubated, and all three patients were preterm with GA 26–31 weeks.

Screening and microbiological analyses

The outbreak strain was susceptible to all antibiotics tested (aztreonam, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin, imipenem, gentamicin, meropenem, piperacillin/tazobactam and trimethoprim–sulfamethoxazole) except ampicillin. Since screening could not be based on antimicrobial resistance characteristics, a culture-based screening method using SCAI was adapted and used in the initial stage of the outbreak (Fig. 1). Using this method, three cases of *K. oxytoca* carriage were detected. Culture-based screening, however, is labour-intensive, partly due to lack of specificity of the selective agar. We therefore developed a *K. oxytoca*-specific real-time PCR which displayed better sensitivity (data not shown) and thus enabled a more frequent, large-scale screening approach from January 2017 (Fig. 1). After implementation of the PCR, 14 additional patients were found to be colonised with the outbreak strain.

Initially, genotyping of isolates was performed using PFGE, and all *K. oxytoca* isolates were also subjected to WGS (Fig. 2). The results of PFGE and WGS were concordant in all isolates investigated. *In silico* multilocus sequence typing of the outbreak strain identified a new sequence type, ST-179, with two new alleles (*infB* 36 and *phoE* 66). Based on these results, we were able to design a fast, *infB* allele-specific PCR assay indicative of the outbreak strain (Table 2), which was useful in the late phase of the outbreak.

Screening of faecal samples from women admitted to the maternity ward (for seven days or longer) by real-time PCR revealed that one in 25 women (4%) were colonised with *K. oxytoca*. However, we were unable to isolate and genotype *K. oxytoca* in pure culture from this individual, most likely due to low bacterial load (high CT value in real-time PCR). The *infB* allele-specific real-time PCR was however negative, indicating that this was not the outbreak strain.

Klebsiella oxytoca that did not belong to the outbreak genotype were isolated from faecal specimens of 12 neonates (NICU 1–12) admitted to the NICU during the study period (NICU 1–11 shown in Fig. 2).

One *K. oxytoca* outbreak strain was found in breastmilk (S2). The outbreak strain was not detected in any of the environmental specimens (Fig. 2). Two specimens from one of the sinks in the department sampled two weeks apart grew *K. oxytoca* isolates (E1a and E1b; Fig. 2) of a similar genotype, but unrelated to the outbreak strain. Of interest is also the finding that three *K. oxytoca* isolates from neonates were of the same genotype but different from the outbreak strain (NICU7-9; Fig. 2).

Infection control measures

A large bundle of infection control measures was implemented to contain the outbreak (Table 1, Fig. 1). Despite the instigation of the first bundle of infection control measures in summer 2016, there were five new cases (two with symptomatic infection and three colonised) during the autumn. Implementation of the real-time PCR for detection in January 2017, which enabled rapid and more extensive assessment of the outbreak, prompted another more extensive bundle of infection control measures including rapid isolation and cohorting of colonised and infected patients. This led first to identification of 14 new cases (one infection and 13 colonised) in January–April 2017, probably aided by the better sensitivity of the PCR method compared to the selective culture. The combination of better screening methods and the second bundle of infection control measures likely contributed to there being no new cases detected after April 2017, despite weekly screening twice a week of all neonates. Surveillance screening by PCR was performed until 31 December 2017.

DISCUSSION/CONCLUSION

We report an outbreak of an antibiotic-sensitive, but virulent *K. oxytoca* strain in a level III NICU. Nosocomial outbreaks are often detected and reported because of an increased incidence rate and/or expression of specific antibiotic resistance determinants rendering the outbreak strain more easily detectable. In this outbreak, it was the clustering of a relatively uncommon pathogen which first raised suspicion of transmission among neonates in the NICU. Subsequently, the identification of genotypically

similar *K. oxytoca* isolates strengthened the suspicion of an ongoing outbreak leading to a more in-depth investigation.

The present report clearly demonstrates the crucial role of adequate microbiological methods in elucidating the real extent of transmission in a NICU. First, the establishment of a selective method for culture of *K. oxytoca* enabled screening of patients and environmental specimens. However, due to the large workload of culture and identification by this method, it was not possible to perform large and frequent screening analyses. Development of a *K. oxytoca*-specific real-time PCR enabled more extensive and frequent screenings, leading to weekly screening twice a week of all admitted patients. This approach also revealed the extent of the outbreak, which again led to more drastic infection control measures being instigated, in particular cohort isolation. Real-time PCR is capable of providing results within a few hours, and a positive result will lead to earlier isolation of the patient thereby reducing exposure time to other patients. Due to infrequent screenings, as well as the use of a less sensitive screening approach in 2016, it was not possible to determine the exact time of first faecal colonisation of patients admitted to the NICU before 2017. The time of first faecal colonisation of patients could however be more precisely determined from January/February 2017 when screening by real-time PCR was conducted more frequently.

Genotyping is of pivotal importance for assessment of transmission of a particular strain in an outbreak setting. Initial PFGE analysis that subsequently was supplemented with WGS produced concordant results with respect to inclusion of outbreak strains and exclusion of unrelated *K. oxytoca* isolates. Data from WGS also allowed construction of an *infB* 36 allele-specific real-time PCR which was used in the late phase of the outbreak and in the follow-up period, allowing rapid exclusion of cases when *K. oxytoca* isolates were negative in the allele-specific PCR. We thus consider WGS to be an important tool in an outbreak elucidation, providing the opportunity to design specific and fast molecular tests tailored to the situation.

Cohorting of infants has proven useful in reducing healthcare-associated infections and colonisation in neonatal units (23). In this outbreak, increased workload and patient crowding were considered important risk factors, and rapid detection of recently colonised patients led to swift allocation to the appropriate cohort, which we consider effective means for reducing the spread of the pathogen. Combined with nursing personnel exclusively assigned to specific cohorts each shift, we believe these measures were critical in controlling the outbreak.

Based on the investigations reported in this study, it is not possible to determine the source of this outbreak. However, we consider carriage by hands of the healthcare workers and/or family members of patients as possible mechanisms of continued transmission of the pathogen. This assumption is supported by data displayed in Figure 1, showing there were neonates colonised with *K. oxytoca* of the outbreak genotype in the NICU at all times from April 2016 until April 2017 serving as a potential reservoir. The outbreak subsided after institution of screenings twice a week with *K. oxytoca*-specific real-time PCR, rapid isolation and cohorting.

Although it is not possible to determine the relative effect of each infection control measure, we believe that the combined effect of improved screening and infection control measures was important to control the outbreak.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

FUNDING

This work did not receive any specific funding.

References

1. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998; 11(4): 589–603.
2. Sahly H, Podschun R. Clinical, bacteriological, and serological aspects of Klebsiella infections and their spondylarthropathic sequelae. *Clin Diagn Lab Immunol* 1997; 4(4): 393–9.
3. Berthelot P, Grattard F, Patural H, Ros A, Jelassi-Saoudin H, Pozzetto B, et al. Nosocomial colonization of premature babies with *Klebsiella oxytoca*: probable role of enteral feeding procedure in transmission and control of the outbreak with the use of gloves. *Infect Control Hosp Epidemiol* 2001; 22(3): 148–51.
4. Jeong SH, Kim WM, Chang CL, Kim JM, Lee K, Chong Y, et al. Neonatal intensive care unit outbreak caused by a strain of *Klebsiella oxytoca* resistant to aztreonam due to overproduction of chromosomal beta-lactamase. *J Hosp Infect* 2001; 48(4): 281–8.
5. Tullus K, Ayling-Smith B, Kuhn I, Rabsch W, Reissbrodt R, Burman LG. Nationwide spread of *Klebsiella oxytoca* K55 in Swedish neonatal special care wards. *APMIS* 1992; 100(11): 1008–14.
6. Beaugerie L, Metz M, Barbut F, Bellaiche G, Bouhnik Y, Raskine L, et al. *Klebsiella oxytoca* as an agent of antibiotic-associated hemorrhagic colitis. *Clin Gastroenterol Hepatol* 2003; 1(5): 370–6.
7. Hogenauer C, Langner C, Beubler E, Lippe IT, Schicho R, Gorkiewicz G, et al. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. *N Engl J Med* 2006; 355(23): 2418–26.
8. Gastmeier P, Loui A, Stamm-Balderjahn S, Hansen S, Zuschneid I, Sohr D, et al. Outbreaks in neonatal intensive care units - they are not like others. *Am J Infect Control* 2007; 35(3): 172–6.
9. Ayan M, Kuzucu C, Durmaz R, Aktas E, Cizmeci Z. Analysis of three outbreaks due to *Klebsiella* species in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2003; 24(7): 495–500.
10. Mavroidi A, Liakopoulos A, Gounaris A, Goudesidou M, Gaitana K, Miriagou V, et al. Successful control of a neonatal outbreak caused mainly by ST20 multidrug-resistant SHV-5-producing *Klebsiella pneumoniae*, Greece. *BMC Pediatr* 2014; 14: 105.

11. Clements A, Halton K, Graves N, Pettitt A, Morton A, Looke D, et al. Overcrowding and understaffing in modern health-care systems: key determinants in meticillin-resistant *Staphylococcus aureus* transmission. *Lancet Infect Dis* 2008; 8(7): 427–34.
12. Jefferies JM, Cooper T, Yam T, Clarke SC. *Pseudomonas aeruginosa* outbreaks in the neonatal intensive care unit–a systematic review of risk factors and environmental sources. *J Med Microbiol* 2012; 61(Pt 8): 1052–61.
13. Van Kregten E, Westerdaal NA, Willers JM. New, simple medium for selective recovery of *Klebsiella pneumoniae* and *Klebsiella oxytoca* from human feces. *J Clin Microbiol* 1984; 20 (5): 936–41.
14. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* 1995; 33(3): 551–5.
15. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33(9): 2233–9.
16. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012; 19(5): 455–77.
17. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014; 30(14): 2068–9.
18. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, et al. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015; 31(22): 3691–3.
19. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol Biol Evol* 2009; 26(7): 1641–50.
20. Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One* 2010; 5(3): e9490.
21. Rambaut A. FigTree. 2016-10-04 ed. Available at: http://www.google.com/url?q=http%3A%2F%2Ftree.bio.ed.ac.uk%2Fsoftware%2Ffigtree%2F&sa=D&sntz=1&usg=AFQjCNHLWieszKe_rWLU_tzBrivx9OfC5g
22. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016; 33(7): 1870–4.
23. Pammi M, Davis RJ, Gordon A, Starke J. Infant isolation and cohorting for preventing or reducing transmission of healthcare-associated infections in neonatal units. *Cochrane Database Syst Rev* 2016; (12): 24.
24. Pittet D, Allegranzi B, Boyce J, World Health Organization World Alliance for Patient Safety First Global Patient Safety Challenge Core Group of Experts. The World Health Organization Guidelines on Hand Hygiene in Health Care and their consensus recommendations. *Infect Control Hosp Epidemiol* 2009; 30(7): 611–22.