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Outbreaks by *Klebsiella oxytoca* in neonatal intensive care units: Analysis of an outbreak in a tertiary hospital and systematic review



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ABSTRACT

Objective: *Klebsiella oxytoca* can cause nosocomial infections, affecting vulnerable newborns. There are few studies describing nosocomial outbreaks in the neonatal intensive care units (NICU). In this study, a systematic review of the literature was carried out to know the main characteristics of these outbreaks and the evolution of one is described.

Methods: We conducted a systematic review in the Medline database up to July 2022, and present a descriptive study of an outbreak with 21 episodes in the NICU of a tertiary hospital, between September 2021 and January 2022.

Results: 9 articles met the inclusion criteria. The duration of outbreaks was found to be variable, of which 4 (44.4%) lasted for a year or more. Colonization (69%) was more frequent than infections (31%) and the mortality rate was 22.4%. In studies describing sources, the most frequent was the environmental origin (57.1%). In our outbreak there were 15 colonizations and 6 infections. The infections were mild conjunctivitis without sequelae. Molecular typing analysis made it possible to detect 4 different clusters.

Conclusions: There is an important variability in the evolution and results of the published outbreaks, highlighting a greater number of colonized, use of PFGE (pulsed-field gel electrophoresis) techniques for molecular typing and implementation of control measures. Finally, we describe an outbreak in which 21 neonates were affected with mild infections, resolved without sequelae and whose control measures were effective.

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Brotos por *Klebsiella oxytoca* en unidades de cuidados intensivos neonatales: análisis de un brote en un hospital de tercer nivel y revisión sistemática

RESUMEN

Objetivos: *Klebsiella oxytoca* puede causar infecciones nosocomiales, afectando a recién nacidos vulnerables. Existen escasos trabajos que describan brotes nosocomiales en las unidades de cuidados intensivos neonatales (UCIN). En este estudio se realiza una revisión sistemática de la literatura para conocer las características principales de estos brotes y se describe la evolución de uno.

Métodos: Se realizó una revisión sistemática, en la base de datos Medline hasta julio de 2022, y un estudio descriptivo de un brote con 21 episodios en la UCIN de un hospital de tercer nivel, desde septiembre 2021 a enero 2022.

Palabras clave:

Klebsiella oxytoca

Neonatos

Brote

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Resultados: 9 artículos cumplían los criterios de inclusión. Se encontró que la duración de los brotes fue variable, de los que 4 (44,4%) se prolongaron durante un año o más. Las colonizaciones (69%) fueron más frecuentes que las infecciones (31%) y la tasa de mortalidad fue del 22,4%. En los estudios que describen las fuentes, lo más frecuente fue el origen ambiental (57,1%). En nuestro brote hubo 15 colonizaciones y 6 infecciones. Las infecciones fueron conjuntivitis leves sin secuelas. El análisis mediante tipado molecular permitió detectar 4 clústeres diferentes.

Conclusiones: Existe una variabilidad importante en la evolución y resultados de los brotes publicados, destacando un mayor número de colonizados, uso de técnicas de PFGE (electroforesis en campo pulsado) para tipado molecular e implantación de medidas de control. Finalmente, describimos un brote en el que se afectaron 21 neonatos con infecciones leves, resueltas sin secuelas y cuyas medidas para el control de este resultaron eficaces.

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Introduction

Klebsiella spp. is ubiquitous in nature, with rates of colonisation increasing in the hospital setting.¹ This microorganism can cause nosocomial infections and is the most common cause of nosocomial outbreaks in neonatal intensive care units (NICU).² *Klebsiella pneumoniae* complex and *Klebsiella oxytoca* complex (KO) are important in humans.³ KO is a human commensal, but it has been described as an opportunistic pathogen causing various infections.^{4,5} It has also been documented as being the cause of outbreaks, most often involving environmental sources.⁶ On the other hand, neonates in the NICU are vulnerable to the acquisition of nosocomial infections, often in the form of outbreaks with enabling factors, and controls are very important.^{7–10} KO can acquire extended-spectrum β -lactamases (ESBLs) and carbapenemases entailing outbreaks that put hospitalised patients at higher risk.^{5,6} Thus, it is essential to implement infection control measures, such as reinforcing hand hygiene, screening to identify the extent of the outbreak and cohort isolation of infected/colonised patients.¹¹

Although KO can cause nosocomial infections, there are no articles that review outbreaks in the NICU. In this work, a systematic review of the literature has been carried out to determine the number, origin and duration of infections, proportions of those infected and colonised, most frequent infections and mortality rates. In addition, the evolution of an outbreak that took place in the NICU of a tertiary hospital is described.

Methods

Systematic review

PRISMA guidelines have been followed (<https://prisma-statement.org/>) using the MEDLINE database, through PubMed®. The terms (*Klebsiella oxytoca* AND outbreak) were used. The search and selection of articles was carried out by two authors. The inclusion criteria were: works published up until 1 July 2022 on the description of outbreaks due to KO in NICUs published in English or Spanish that had at least the following minimum variables: duration of the outbreak and number of patients affected, and types of infection, if present. The references listed in the studies were also reviewed to reduce the number of losses. There was no rejection of articles by language (Fig. 1). Items from the ORION statement for transparent reporting of outbreaks were used to analyse risk of bias and individual study results.

Outbreak analysis

The outbreak that took place between September 2021 and January 2022 was investigated. Outbreak was defined as the association of two or more cases of healthcare-associated infection (HAI) by KO in patients admitted to the NICU. A case of infec-

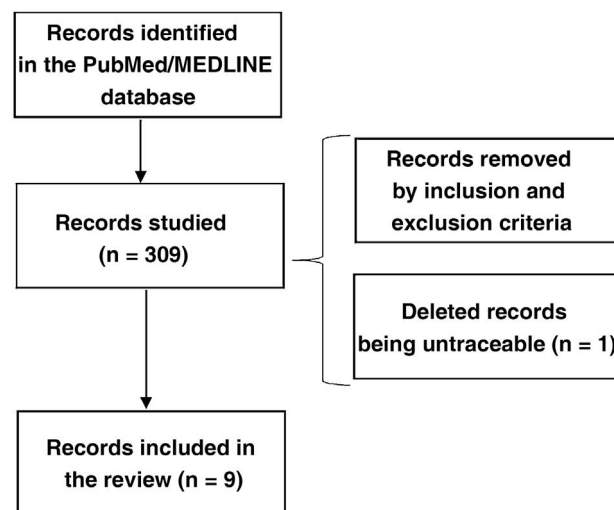


Figure 1. Search for articles diagram. The length of the bars is equivalent to the duration of stay in the NICU in weeks.

tion associated with the outbreak was determined as a patient admitted with a clinical sample with KO and signs and/or symptoms of HAI in that location, while a case of colonisation associated with the outbreak was described as an admitted patient, with KO isolated in a biological sample but no clinical signs of infection. The following variables were collected for each infected/colonised neonate: sex, gestational age, birth weight, dates of admission and discharge, presence of antibiotics at birth, central or peripheral venous/arterial catheter, intubation and duration days of each, length of time in the NICU, clinical course, date of first positive culture, colonisation, types of KO infection, treatment received, co-infection by other microorganisms, and location of the neonate at the time of KO clinical specimen collection.

Site

The NICU, located on the fifth floor of the Maternal and Child Hospital of the Hospital Universitario Virgen de las Nieves [Virgen de las Nieves University Hospital], has an area for basic care (20 cots), an area for intermediate care (10 cots) and a third area for intensive care (8 cots). In this unit, infants are cared for according to their needs from the moment of birth.

Outbreak control measures

To monitor the outbreak, prevention measures (organisational measures, epidemiological surveillance, measures to prevent the transmission of the microorganism) and control (information, training and environmental measures) from the document

Apoyo metodológico para el abordaje integral de brotes nosocomiales [Methodological Support for a Comprehensive Approach to Nosocomial Outbreaks] and the *Protocolo de vigilancia y control de brotes por IRAS* [HAI Outbreak Surveillance and Control Protocol] of the Ministry of Health of Andalusia were implemented.^{12,13}

Microbiological study of clinical isolates associated with the outbreak by the PIRASOA [Programa Integral de Prevención y Control de las Infecciones relacionadas con la Asistencia Sanitaria y el Uso Apropiado de Antimicrobianos (Comprehensive Programme for the Prevention and Control of Healthcare-Associated Infections and Appropriate Use of Antimicrobials)] Programme

The isolates were identified using MALDI-TOF (Biotyper, Bruker Daltonics, Billerica, MA, USA). Antimicrobial susceptibility testing was conducted with microdilution (MicroScan WalkAway, Beckman-Coulter, Brea, CA, USA) and interpreted according to the EUCAST cut-off points. (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0.Breakpoint.Tables.pdf). Clonal relationships among isolates were assessed with pulsed-field gel electrophoresis (PFGE). Complete digestion of chromosomal DNA was performed with *Xba*I. The resulting restriction fragments were separated on the CHEF DR-II system (Bio-Rad, Alcobendas, Madrid, Spain) with 1% agarose gel. Gels were stained with ethidium bromide, visualised under ultraviolet light, and photographed on a Gel Logic 200 Automated Imaging System (Kodak, Rochester, NY, USA). Band pattern conversion, normalisation and analysis was performed using BioNumerics 8.1 software (Applied Maths, Jollyville Rd., Austin, TX, USA). Band position tolerance and optimisation were set to 1%. An unweighted pair group method with arithmetic mean was used to generate a dendrogram, and dendrogram analysis was used to measure genetic similarity between isolates. Isolates that differed by two or more bands were considered different.¹⁴ Isolates with one or more band differences in PFGE assays were selected for sequencing. At least one isolate from each cluster was analysed by sequencing (Illumina®). Open raw read fragments were assembled *de novo* using the CLC Genomics Workbench, v9.1 (Qiagen Iberia, Las Rozas de Madrid, Madrid, Spain) software. Resistance determinants were detected using the ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder>) and CARD (<https://card.mcmaster.ca/>) databases and the MLST was identified using the MLST finder 2.0 database (<https://cge.cbs.dtu.dk/services/MLST>).

ORION declaration

The ORION statement guidelines were followed to ensure transparency in outbreak reporting (https://www.ucl.ac.uk/drupal/site-antimicrobial-resistance/sites/antimicrobial-resistance/files/checklist_authors.pdf).

Ethical approval

The Granada Research Ethics Committee approved the project on 29 July 2022 with reference code 1385-N-22.

Results

Systematic review

The search returned 309 publications. Ten met the specified inclusion criteria, and nine were included for the review, as one could not be located (Ethiop Med J. 1997;35(3):177–83). The characteristics of these nine outbreaks are shown in Table 1. All occurred on the European continent, except for one in South Korea. There

were two outbreaks in Germany (22.2%). KO presented some type of resistance in five studies (55.5%): in three outbreaks, there was the production of ESBL (33.3%), one being CTX-M-15²²; in one, there was the production of VIM-type carbapenemase (11.1%)²¹ and in another chromosomal β -lactamase with resistance to aztreonam (11.1%).¹⁸ The duration of the outbreak was variable, from two months ($n = 3$; 33.3%) to one year or longer ($n = 4$; 44.4%), the longest being from 1996 to 1999. The remaining outbreaks ($n = 2$; 22.2%) lasted four and nine months. Regarding the spread, in three of the studies (33.3%) KO spread to other hospital wards.^{16,17,22} Considering those affected, we found 193 neonates who were colonised or infected, of which 58 episodes were infections, 129 were colonisations, and in six neonates, it was not documented. In six studies (66.7%), the presence of colonisation and infection was described; in one outbreak (11.1%), there was only infection¹⁶ and in another (11.1%) only colonisation.²² There was one episode in which it was not described. Colonisations ranged from one to 62 and infections ranged from one to 24. Of the documented infections, the most frequent were bacteraemia (78.6%), followed by conjunctivitis (7.1%), pneumonia, peritonitis, unspecified invasive infections (5.4%), and urinary tract infection (1.8%). In the 58 infections described there were 13 deaths (total mortality rate of 22.4%). One of the deaths was reported as due to a cause other than infection. The clinical course of the neonates was not reported in three studies (33.3%). Of the origins of the outbreaks that were documented ($n = 7$; 77.8%), the newborns themselves were considered probable origins in two (28.6%), and in four (57.1%), the most frequent was environmental origin (disinfectant solution, washing machine, humidifiers and enteral nutrition tube). In 14.3% the most probable origin of the outbreak could not be established.⁶

Regarding the molecular typing analysis, it was not described for all the outbreak episodes. Of those that were described ($n = 6$; 66.7%), the techniques most frequently used were PCR and PFGE. The lineage type found in each study was reported in all but one; thus, of the total, 50% of the studies described the same lineage, 25% were three different strains and two different ones in the remaining 25%. Finally, we found studies in which there was no record of applying measures to control the outbreak (22.2%), while the rest of the studies did report the control measures that were carried out.

Outbreak analysis

Outbreak development

Surveillance of HAIs in the NICU is carried out by the Hospital's Servicio de Medicina Preventiva y Salud Pública [Preventive Medicine and Public Health Service] (MP y SP). On 27 September 2021, two cases of conjunctivitis due to KO in neonates were detected. Reviewing the previous microbiological isolates in that unit, the existence of a third neonate with conjunctivitis due to the same microorganism that started on 13 September was noted. Considering that all the cases were admitted to the same NICU module and occurred in the same period (spatiotemporal association), MP y SP declared the outbreak to the Andalusian Epidemiological Surveillance System. An Improvement Group was established to address the outbreak that same day. From then on, with the aim of discovering new patients affected by KO in the neonatology unit, an active search for infected and colonised patients was ongoing.

Thus, aiming to quantify the magnitude of the outbreak and control its expansion, rectal smears were performed weekly on all admitted neonates and during this investigative process, six patients with nosocomial conjunctivitis due to KO and 15 colonised patients were identified (Fig. 2). Finally, after at least four weeks without detection of new cases of infected or colonised patients, the outbreak was concluded on 20 January 2022.

Table 1Main characteristics in the description of outbreaks due to *Klebsiella oxytoca* in the neonatal ICU.

Author (year of publication)	Country	Antibiotic resistance	Duration of outbreak	Other hospital wards affected	No. affected (neonates only)	Types of infection	Clinical course	Source (origin of the outbreak)	Genotyping Technique (lineage)	Control measures
Morgan et al. ¹⁵ (1984)	United Kingdom	Gentamicin	May 1981–January 1982	No	74 neonates (12 infected and 62 colonised)	10 bacteraemias 2 peritonitis	8 deaths	Probable neonates	NS	Yes
Irwin Reiss et al. ¹⁶ (2000)	Germany	–	October 1996–March 1999	Yes	24 neonates (24 infected)	24 bacteraemias	2 deaths	Probable disinfectant solution	Plasmid analyses and 16 S rRNA gene sequence (same lineage)	Yes
Berthelot et al. ¹⁷ (2001)	France	Amoxicillin, ticarcillin and piperacillin.	June 1996–February 1997	Yes	18 neonates (one infected and 17 colonised)	1 bacteraemia	1 death	Probable enteral nutrition tube	NS (2 different lineages)	Yes
Jeong et al. ¹⁸ (2001)	South Korea	Chromosomal β -lactamase	November–December 1997	No	6 neonates (3 infected and 3 colonised)	2 conjunctivitis 1 urine infection	NS	Probable humidifiers	PCR-PFGE (same lineage)	NS
Ayan et al. ¹⁹ (2003)	Turkey	ESBL (20%)	May–June 2000	No	10 neonates (9 infected and 1 colonised)	9 bacteraemias	NS	NS	AP-PCR (3 different lineages)	Yes
Grisold Aj et al. ²⁰ (2009)	Austria	ESBL	July–August 2007	No	6 neonates (NS)	NS	NS	NS	Rep-PCR and PFGE (2 different lineages)	NS
Herruzo et al. ²¹ (2017)	Spain	VIM carbapenemase	February 2014–May 2014	No	20 neonates (4 infected and 16 colonised)	3 pneumonias 1 conjunctivitis	1 death (another cause)	Probable neonates	NS (3 different lineages)	Yes
Ronning TG et al. ⁶ (2018)	Norway		April 2016–April 2017	No	22 neonates (5 infected and 17 colonised)	1 peritonitis 1 conjunctivitis 3 invasive infections (bacteraemia and/or pneumonia)	1 death	Not found	PFGE- WGS: (same lineage) ST-179	Yes
Schmithausen RM et al. ²² (2019)	Germany	ESBL	April 2012–May 2013	Yes	13 neonates (13 colonised)	No infection	0 deaths	Probable washing machine	PCR-PFGE-MLST (same lineage) PFGE type 00531/ST201 ESBL producers	Yes

AP-PCR, arbitrarily primed polymerase chain reaction; ESBL, extended-spectrum beta-lactamases; MLST, multilocus sequence typing; NS, not stated; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; Rep-PCR, polymerase chain reaction based on repetitive element sequences; WGS, whole-genomic sequencing.

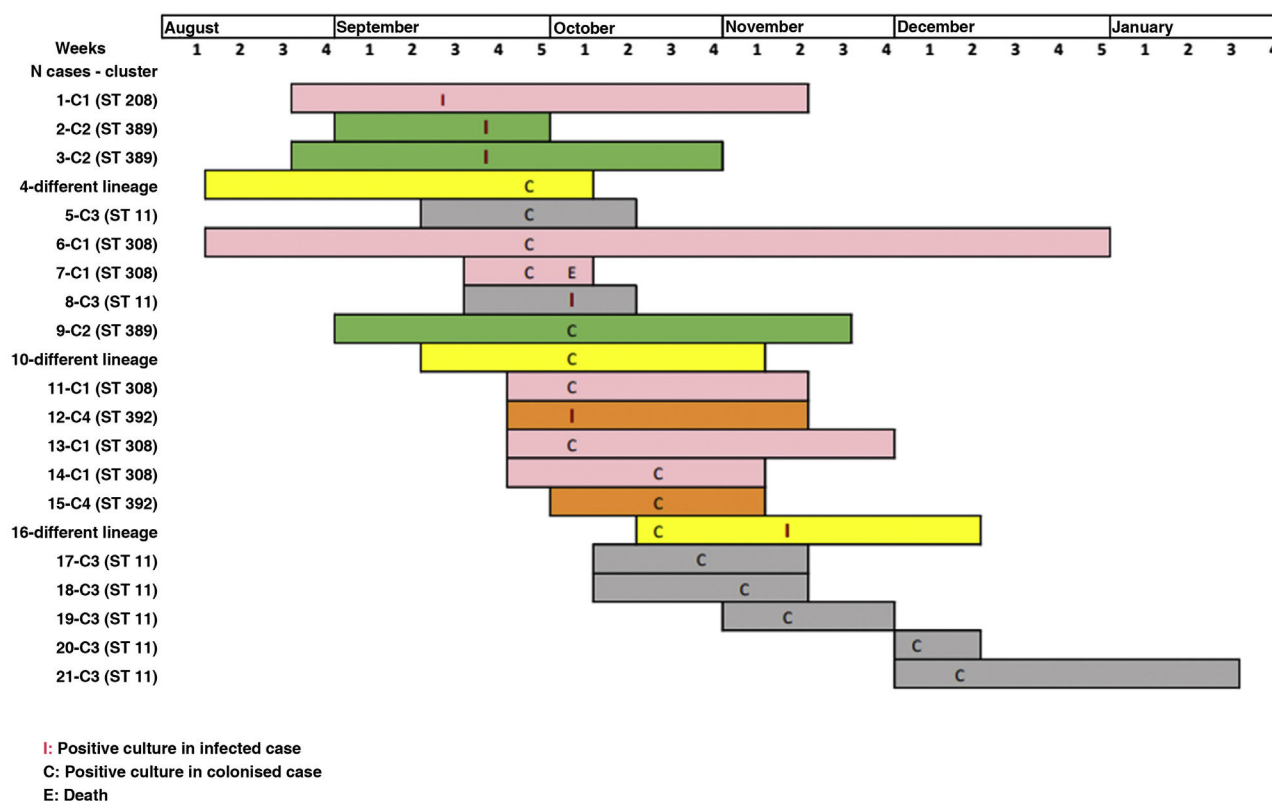


Figure 2. Epidemic curve of cases from September 2021 to January 2022.

Clinical-epidemiological research

During the outbreak period, 128 patients in the NICU were analysed, 21 (16.4%) of whom had a clinical sample that was positive for KO during their admission. Of these 21 neonates (Table 2), 13 (61.9%) were male, with a mean gestational weight of 1,468.80 g (range: 450–3,000 g and SD: 571.8) and age of 31.3 weeks (range: 0–40 weeks and SD: 3.9). The mean length of stay in the NICU was 45.5 days (range: 10–135 and SD: 29.6). Only one neonate died from a cause unrelated to KO infection. Regarding the location of the patients at the time of microbiological isolation, 11 (52.4%) were in the intensive care module, three (14.3%) were in the intermediate care module and seven (33.3%) were in the basic care module.

Regarding the isolates detected from the 21 cases, 15 (71.4%) were colonisations and six (28.6%), infections. All the infections identified were mild conjunctivitis treated with gentamicin eye drops (Table 2).

Microbiological investigation

Antibiotic susceptibility was studied in the conjunctival isolates, which shared the same profile: susceptibility to piperacillin-tazobactam, cefotaxime, cefepime, ertapenem, imipenem, meropenem, aztreonam, ciprofloxacin, gentamicin, tobramycin, amikacin, fosfomycin, and trimethoprim/sulfamethoxazole.

Molecular typing analysis

The 21 isolates of *K. oxytoca* were sent to the reference laboratory for analysis by PFGE and sequencing. Following their analysis, four different clusters were identified: ST11 (7; 33.3%), ST308 (6; 28.6%), ST389 (3; 14.3%) and ST392 (2; 9.5%), and in three neonates the pulsotypes were different from each other and different from all the identified clusters (Fig. 3). The analysis allowed us to conclude that two cases of conjunctivitis and one colonised patient belonged

to the same lineage (ST389, cases 2, 3 and 9). Cases 1, 6, 7, 11, 13 and 14 belonged to clone ST308; cases 5, 8, 17, 18, 19, 20 and 21 were part of clone ST11, while cases 12 and 15 corresponded to ST392. Finally, cases 4, 10 and 16 were different from each other and from the rest of the cases. Thus, four different transmission chains were found in the outbreak.

Outbreak control measures

Surveillance of HALs is carried out in this hospital and general control measures, such as the support of the hospital management, staff education with periodic and onboarding training, appropriate use of antibiotic therapy, epidemiological surveillance and the use of and adherence to transmission-based precautions, are implemented. With the detection of this outbreak, measures were added to those of the Programa de vigilancia y control de la infección nosocomial [Nosocomial Infection Surveillance and Control Programme] in the neonatology unit (Table 3).

Discussion

Despite the fact that, according to one article,² *Klebsiella* is the leading cause of outbreaks in NICUs, the majority due to *K. pneumoniae*, in this study, the paucity of reported outbreaks caused by KO in NICUs is striking. Nine episodes in developed, high-income, and middle-income countries were included in this systematic review, according to the classification by the World Data Bank,²³ as well as in another review on outbreaks of *Pseudomonas aeruginosa* in NICUs²⁴ and another on NICU outbreaks.²⁵ Regarding resistance in KO, it appears that there is an increase in outbreaks closest to the present day, related to the increase in multidrug-resistant microorganisms.²⁶ This fact makes it difficult to treat infections and escalates the consequences. Regarding the proportion of those

Table 2
General characteristics of neonates hospitalised in the NICU (n = 21).

Sex	Gestational age (weeks)	Birth weight	Admission time (days)	Infection/colonisation		Infection Tx.		Location		Clinical course			
				F (n-%)	(Median, IQR)	Infection (n-)	Colonisation (n-%)	Gentamicin eye drops (n-%)	No Tx. (n-%)	Basic (n-%)	Intermediate (n-%)	Intensive (n-%)	Cured (n-%)
M (n-%)	(13–61.9)	(32, 29–33)	(1,460, 1,100–1,700)	(39.25–59)	(6–28.6)	(15–71.4)	(5–83.3)	(1–16.7)	(5–23.8)	(5–23.8)	(11–52.4)	(20–95.2)	(1–4.8)

M, male; F, female; IQR, interquartile range; Tx, treatment

affected, a high number was observed in most of them. However, in alignment with other reviews,^{7,25} the number of colonised patients is greater than the number of infected patients, which supports the idea that colonised neonates are one of the main reservoirs and the hands of health personnel are one of the main vectors.^{27,28} Mortality in the review was highly variable, with a total rate of 22.4%, while in the review on outbreaks due to ESBL-producing Enterobacteriaceae the rate was 16%.²⁵ Although in many instances the origin of the outbreaks cannot be established, the probability of an environmental origin, such as washing machines, humidifiers or disinfectants, is noteworthy and corroborated by scientific evidence.⁶ Most of the molecular analysis consisted of PCR-PFGE, with variability in the lineages present, as occurs in other outbreaks due to gram-negative bacteria.²⁹ Regarding control measures, at least one was carried out in all the outbreaks in which they are documented, some of the most common being reinforcing hand hygiene and the cohort isolation of infected/colonised neonates. In this way, outbreaks in the NICU due to KO have important implications, affecting a significant number of neonates who acquire the infection or colonisation, and may act as a reservoir for its spread.

In this work we studied a polyclonal outbreak due to KO that involved 21 neonates from the NICU of a tertiary level hospital. Some risk factors for the acquisition of nosocomial infections in vulnerable neonates in the NICU, such as low birth weight (<1,500 g), prematurity, use of invasive devices, antibiotic therapy, and length of stay in the NICU, have been well identified in the literature.⁸ In this outbreak, 57.1% of neonates had low birth weight, 90.5% were premature, and 95.3% had some kind of invasive device for at least 24 h. Regarding mortality rates, their variability is observed in the review. In our outbreak we only had one death due to another cause. Regarding the cases that made up the outbreak, we found 15 colonisations and six infections, similar to the other publications, with more colonised cases. All infections were mild (conjunctivitis) and resolved satisfactorily. The epidemiological surveillance measures (weekly screening of those admitted to the unit, active search for new cases and sending the isolates to the reference laboratory) were very useful. Molecular typing confirmed that the outbreak was comprised of four different chains of transmission belonging to different clones, as in the outbreaks included in the review.

In outbreaks it is possible to identify probable sources of environmental spread, such as washing machines,²⁰ disinfectants,¹⁶ multi-dose vials³⁰ or enteral tubes.¹⁷ However, in some outbreaks the origin remains uncertain. No environmental study was carried out for our outbreak, since the measures focused on newborn screening and ongoing training for NICU staff, as well as those described in Table 3. In fact, although it is not possible to determine a single measure that had the greatest influence on the reduction of the outbreak, it is likely that it was due to the measures as a whole. Even though the origin of this outbreak is not clear, the most likely explanation is that colonised neonates acted as the main reservoir and transmission to others occurred through the hands of staff, which has been described as one of the main routes of transmission for outbreaks,²⁶ since the cases are spaced out in time and this does not suggest a common origin.

The outbreak was concluded on 20 January 2022, after at least four weeks had passed without new cases of infection or colonisation. As of the end of this outbreak, measures were stipulated to prevent further outbreaks, and if HAI did arise, they would be detected early. These were the performance of routine screening through colonisation studies (rectal smears) every two weeks in a situation without an outbreak to look for gram-negative bacteria other than *Escherichia coli*; use of single-dose vials for eye care, and reinforcing hand hygiene by the NICU and MP y SP. Since implementing these measures, no further outbreaks have been reported in the NICU to date.

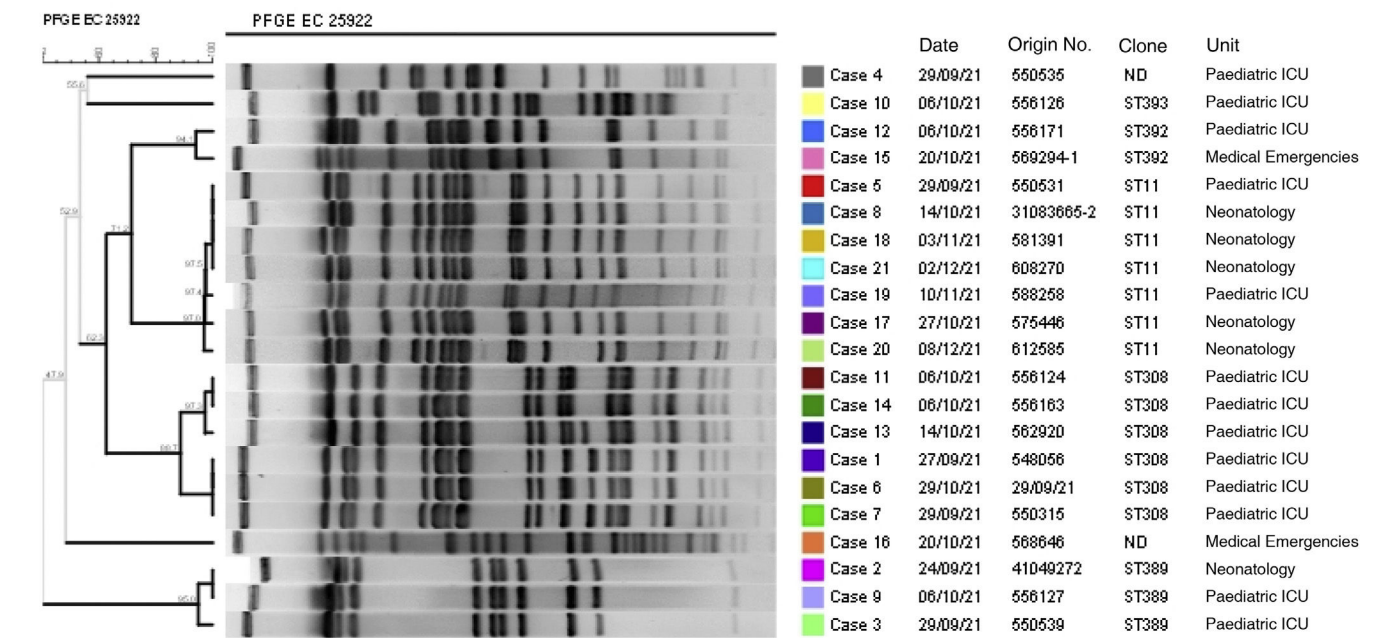


Figure 3. Phylogenetic tree of isolates.

Table 3
Outbreak control measures.

Organisational-type measures
Creation of a Multidisciplinary Improvement Group to address this outbreak (27/09/2021)
Work session of the Multidisciplinary Improvement Group: analysis of the situation (18/10/2021)
Epidemiological surveillance
Visit to the unit by MP y SP to assess the problem in situ (28/09/2021)
Active search for new cases
Weekly screening of those admitted to the neonatal unit
Sending of isolates related to the outbreak to the reference laboratory
Precautions to prevent transmission of infection
Contact precautions in cases (colonised/infected)
Attention to hand hygiene and proper use of gloves
Follow-up by MP y SP nurses regarding compliance with the standard measures for preventing nosocomial infection, special attention to patient care procedures, cleaning/disinfection of fomites and material, and proper hand hygiene (start 28/09/2021).
Terminal cleaning of affected rooms and modules
Information and training
Training workshops in small groups, including all professional categories, on hand hygiene and proper use of gloves (start 21/10/2021).
Information for relatives about isolation measures
Improvement in the signage of the entrances to the unit
Other measures
Changing taps in unit to be sensor activated
Presence of antiseptic soap with chlorhexidine in all the soap dispensers in the unit, to which the dispenser sensor has been added.
Complete evacuation of the module to proceed with the terminal cleaning/disinfection of the room, including material, shelves and lockers. Subsequently, the disinfection procedure using ultraviolet rays is applied (XENEX MACHINE)

This study has several limitations. On the one hand, although the systematic review is exhaustive, it was carried out in a single database, so there may be a selection bias. On the other hand, there was also an article that could not be accessed. Notably, not all the outbreaks reported the characteristics listed in Table 1, so there may be a bias in the results' scope. It is necessary to homogeneously analyse the appearance of outbreaks in the NICU to collect complete data for systematic reviews.

In conclusion, there is a lack of studies in the literature on KO outbreaks in the NICU. There is significant variability in the evolution and results of KO outbreaks, with similarity regarding the appearance of a greater number of colonised than infected patients, the use of PFGE techniques for molecular typing and the implementation of control measures standing out. Finally, an outbreak

has been described in which 21 newborns were affected with mild infections and for which the measures were effective in controlling it. Implementing the stipulated measures after the outbreak to improve the early detection of HAI and prevent transmission had beneficial consequences, as no more outbreaks have occurred since the one described.

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No funding was received for conducting this study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

- Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998;11:589–603.
- 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;35:172–6.
- Tártara SG. Patógenos emergentes: tercera parte. *Klebsiella pneumoniae* productora de carbapenemasas. Rev Nefrol Diál Trasplant. 2013;33:103–9.
- Yang J, Long H, Hu Y, Feng Y, McNally A, Zong Z. *Klebsiella oxytoca* complex: update on taxonomy, antimicrobial resistance, and virulence. Clin Microbiol Rev. 2022;35(1):e0000621, <http://dx.doi.org/10.1128/CMR.00006-21>.
- Neog N, Phukan U, Puzari M, Sharma M, Chetia P. *Klebsiella oxytoca* and emerging nosocomial infections. Curr Microbiol. 2021;78:1115–23.
- Rønning TG, Aas CG, Støen R, Bergh K, Afset JE, Holte MS, et al. Investigation of an outbreak caused by antibiotic-susceptible *Klebsiella oxytoca* in a neonatal intensive care unit in Norway. Acta Paediatr. 2019;108:76–82, <http://dx.doi.org/10.1111/apa.14584>.
- Johnson J, Quach C. Outbreaks in the neonatal ICU: a review of the literature. Curr Opin Infect Dis. 2017;30:395–403.
- Decembrino L, Maini A, Decembrino N, Maggi I, Lacerenza S. Management of outbreaks in neonatal intensive care units. Early Hum Dev. 2014;90 Suppl. 1:S54–6.
- Fernández-Prada M, Martínez-Ortega C, Santos-Simarro G, Morán-Álvarez P, Fernández-Verdugo A, Costa-Romero M. Brote de *Klebsiella pneumoniae* productora de betalactamasas de espectro extendido en una unidad de cuidados intensivos neonatales: factores de riesgo y medidas de prevención clave para su erradicación en tiempo récord. An Pediatr. 2019;91:13–20.
- Sumer S, Turk Dagı H, Findik D, Arslan U, Aktug Demir N, Ural O, et al. Two outbreaks of ESBL-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. Pediatr Int. 2014;56:222–6, <http://dx.doi.org/10.1111/ped.12234>.
- Montagnani C, Cocchi P, Lega L, Campana S, Biermann KP, Braggion C, et al. *Serratia marcescens* outbreak in a neonatal intensive care unit: crucial role of implementing hand hygiene among external consultants. BMC Infect Dis. 2015;15:11, <http://dx.doi.org/10.1186/s12879-014-0734-6>.
- Ballesteros García L, Díaz Molina C, Figueroa Murillo E, Gallardo García V, Gasch Illescas A, Gómez Olmedo M, et al. Apoyo metodológico para el abordaje integral de brotes nosocomiales: Consejería de Salud. Sevilla: Junta de Andalucía; 2006.
- Grupo de Trabajo de Vigilancia de las IRAS. Red Nacional de Vigilancia Epidemiológica (RENAVE). Protocolo de vigilancia de brotes de Infecciones relacionadas con la asistencia sanitaria (Protocolo-BROTES). Madrid, noviembre de 2016. Revisado en abril 2019.
- 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;33:2233–9, <http://dx.doi.org/10.1128/jcm.33.9.2233-2239>.
- Morgan MEI, Hart CA, Cooke RWI. *Klebsiella* infection in a neonatal intensive care unit: role of bacteriological surveillance. J Hosp Infect. 1984;5:377–85.
- Reiss I, Borkhardt A, Füssle R, Sziegoleit A, Gortner L. Disinfectant contaminated with *Klebsiella oxytoca* as a source of sepsis in babies. Lancet. 2000;356(9226):310, [http://dx.doi.org/10.1016/S0140-6736\(00\)02509-5](http://dx.doi.org/10.1016/S0140-6736(00)02509-5).
- 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;22:148–51.
- 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 β -lactamase. J Hosp Infect. 2001;48:281–8.
- 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;24:495–500.
- Grisold AJ, Zarfel G, Strenger V, Feierl G, Leitner E, Masoud L, et al. Use of automated repetitive-sequence-based PCR for rapid laboratory confirmation of nosocomial outbreaks. J Infect. 2010;60:44–51.
- Herruzo R, Ruiz G, Gallego S, Diez J, Sarria A, Omeñaca F. VIM-*Klebsiella oxytoca* outbreak in a neonatal intensive care unit. This time it wasn't the drain. J Prev Med Hyg. 2017;58(4):E302–7.
- Schmithausen RM, Sib E, Exner M, Hack S, Rösing C, Ciorba P, et al. The washing machine as a reservoir for transmission of extended-spectrum-beta-lactamase (CTX-M-15)-producing *Klebsiella oxytoca* ST201 to newborns. Appl Environ Microbiol. 2019;85(22):e01435–19, <http://dx.doi.org/10.1128/AEM.01435-19>.
- Datatopics.worldbank.org. Available online: <https://datatopics.worldbank.org/world-development-indicators/the-world-by-income-and-region.html> [consultada el 29 de julio de 2022].
- Jefferies JMC, 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;61:1052–61.
- Stapleton PJM, Murphy M, McCallion N, Brennan M, Cunney R, Drew RJ. Outbreaks of extended spectrum beta-lactamase-producing *Enterobacteriaceae* in neonatal intensive care units: a systematic review. Arch Dis Child Fetal Neonatal Ed. 2016;101(1):F72–8.
- Sleiman A, Fayad AGA, Banna H, Matar GM. Prevalence and molecular epidemiology of carbapenem-resistant Gram-negative bacilli and their resistance determinants in the Eastern Mediterranean Region over the last decade. J Glob Antimicrob Resist. 2021;5:209–21.
- Knittle MA, Eitzman DV, Baer H. Role of hand contamination of personnel in the epidemiology of gram-negative nosocomial infections. J Pediatr. 1975;86:433–7.
- Tajeddin E, Rashidan M, Razaghi M, Javadi SSS, Sherafat SJ, Alebouyeh M, et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. J Infect Public Health. 2016;9:13–23.
- Millán-Lou MI, López C, Bueno J, Pérez-Laguna V, Lapresta C, Fuertes ME, et al. Successful control of *Serratia marcescens* outbreak in a neonatal unit of a tertiary-care hospital in Spain. Enferm Infect Microbiol Clin. 2021;40:248–54.
- Mete B, Aybar Bilir Y, Aygun G, Yilmaz M, Urkmez S, Dikmen Y, et al. *Klebsiella oxytoca* outbreak in an intensive care unit: a probable link to common insulin vial use. Anaesth Intensive Care. 2013;41:266–8.