Enterobacter cloacae complex outbreak in a neonatal intensive care unit: multifaceted investigations and preventive measures are needed

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Enterobacter cloacae complex outbreak in a neonatal intensive care unit: multifacet investigations and preventive measures are needed

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List of key words

NICU, outbreak, Enterobacter cloacae, cross-transmission, preterm.

Abstract

We report the investigation to control an *Enterobacter cloacae* complex outbreak in a NICU from November 2020 to February 2021. Pulsed-field gel electrophoresis showed that 5/8 cases were infected with a clonal strain. Breast pumps, shared among mothers in the unit, could have contributed to the spread of the clonal spread.

Background

Neonates are at high risk of nosocomial infection, especially those with extreme prematurity. During a hospitalization in a neonatal intensive care unit (NICU), almost one in ten children will acquire nosocomial infections (1). The *Enterobacter cloacae* complex (ECC) has been often reported in nosocomial outbreaks in NICU (2-4). This report aims to describe the investigations and measures taken to control an ECC outbreak in a NICU. We used the CARE check-list to validate our epidemiological investigation (5).

Definition and usual infection control protocols

Infection was defined as any preterm infant with *E. cloacae* positive clinical sample and clinical symptoms. Colonization was defined as any preterm infant with *E. cloacae* positive sample with no clinical symptoms (clinical sample or systematic rectal swab for digestive colonization).

No systematic screening for multidrug resistant (MDR) bacteria is routinely performed as infections caused by MDR bacteria are rare in our NICU. Contact or droplet isolation measures are taken if a neonate is infected with a MDR according to the French guidelines.

Microbiological methods

Relatedness among all *E. cloacae* isolates was investigated by pulsed-field gel electrophoresis (PFGE), performed according to the manufacturer's instructions (BioRad, Marnes-la-coquette, France). Whole-cell DNA was digested with the SpeI restriction enzyme overnight at 37C. Electrophoresis was performedwith a CHEF DRII apparatus (BioRad) through a 1% agarose gel in 0.5 Triseboratee EDTA buffer. Migration conditions were as follows: temperature, 14C; voltage, 6 V/cm; switch angle, 120 with one linear switch ramps of 2e20 s for 20 h. After migration, gels were stained in a 0.5 mg/mL ethidium bromide solution. PFGE profiles were analyzed using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium) and PFGE results were analyzed according to the criteria of Tenover.

Description of the outbreak

The NICU at Nantes University Hospital, France, is a 14-bed unit (incubators) with 10 single and 4 double rooms, respectively. From November 2020 to February 2021, eight symptomatic neonates had *E. cloacae complex* positive sample. They were mainly isolated from blood culture (n=5/8). The first two cases were twins and the third was hospitalized in the adjacent room. During eight weeks, four more neonates had a nosocomial enterobacter infection, and one was identified colonized in the gut flora and detected by a systematic screening for *E. cloacae* colonization. Cases' maternal and neonatal factors are described in the table. *E. cloacae* was not endemic in the NICU before the outbreak and no clinical sample was identified by the lab database analysis in the two last years. No systematic screening for multidrug resistant bacteria (MDR) digestive colonization is routinely performed as infections caused by MDR are rare in our NICU. Contact or droplet isolation measures are taken if a neonate is infected with a MDR according to the French guidelines.

There were three antibiotic susceptibility profiles in this outbreak: wild-type, wild type, Chromosomal AmpC overproduction, Extended spectrum beta-lactamase production. PFGE evaluation of genetic relationship between all the isolates of ECC is described in Fig 1.

Investigations and control measures

As commensal bacteria of the intestinal tract, *E. cloacae* can be transmitted by contact, such as health care worker's (HCWs) hands or patient-care equipment. The first measure was an intensive cleaning and disinfection of the rooms of the unit. Children with infection or colonization with resistant *E. cloacae complex* were cohorted and contact precautions were used. Observations of practices were carried out during a week to identify potential sources of contamination. HCWs were guided to use personal protective equipment appropriately, especially discarding them before exiting the patient room. As part of the training of professionals on standard precautions, we moved from systematic use of gowns changed once/24 hours/room to a targeted use of gowns, changed at each use, with a strong promotion of hand hygiene. We also suspected the Fortipré® food supplement used for four of the infected premature infants. The results of the microbiological testing of samples of the batches used were negative. We also noted that this type of food supplement, despite the absence of changes in practice of use, can cause digestive hyperosmolarity and facilitate the emergence of pathogenic bacteria. Regarding medical equipment shared among the unit, we noticed that several mothers used the same breast pump and where in charge of its disinfection. The NICU was then equipped with six additional breast pumps in order to dedicate one per room. The disinfection protocol may have been not optimal and biofilm may explain the cross-transmission. The medical staff of others unit, in particular radiologists and physiotherapists who must care neonates in the NICU, have been warned and asked to respect

the measures implemented. They were asked to pay attention to the cleaning of their equipment.

Our first hypothesis was cross-transmission because cases 1, 2 and 3 were in adjacent rooms. To identify if other neonates may have acquired the same bacteria through cross-transmission, we weekly screened all babies with rectal swabs. Only one of 100 neonates screened during the study period (number 8), has a positive ECC stool sample. In order to assess whether cross transmission could explain the outbreak, we performed a pulsed-field gel electrophoresis analysis including all *E. cloacae complex* isolates recovered during the study period (Figure 1). Among all analyzed strains, we observed five chromosomal AmpC overproduction phenotype, four wild phenotype, and two extended-spectrum β-lactamase producer phenotypes. Neonates 1, 2, 3 and 5 were infected with the same clone with chromosomal AmpC overproduction phenotype. Cross-transmission was therefore the least likely hypothesis to explain the others cases identified.

Due to numerous positive blood cultures, attention was paid to the management of invasive devices including central and peripheral venous catheters. No deficiencies in practice were found. Moreover, some articles have suggested parenteral nutrition or multiple-dose medications as a potential source of contamination (6,7). All parenteral nutritions were produced at the hospital pharmacy unit, with a microbiological control before the release. After investigation, none of them was defective.

Regarding antibiotic therapy, the NICU has changed its protocols following another outbreak several months before (*Klebsiella* colonization) and broad-spectrum antibiotics (Third cephalosporin generation) were used more often. This change in antibiotics used, may have selected for resistant bacteria such as ECC. The medical staff of the NICU and antibiotic prescribing paediatricians worked together to initiate an optimized antibiotic use with less

selection pressure on the gut flora. Another possible contributory factor was mothers' exposure to amoxicillin during delivery, that may have played a role in modifying the gut microbiota of newborns, particularly in cases of susceptible strains of *E. cloacae*, but the time to onset of infection was often very short compared to birth.

Interpretation of the investigation

This was a multifactorial outbreak. We did not identify a unique source of contamination.

Cross-transmission was possible and highlighted by comparing the isolates (Fig.1). Four neonates had the same clone. There were two cases, twins, in the same room and one case diagnosed later in the adjacent room. In this situation, a transmission through HCWs is highly probable. Some medical equipment is shared across the unit and could have contributed to the spread of this clonal strain (8), such as breast pumps. The NICU has acquired additional breast pumps, to dedicate one for each room.

As previously reported, preterm neonates involved in this outbreak were very preterm infants with premature and prolonged rupture of membranes' mother (Tab.1). Some of them had a necrotizing enterocolitis, or ventilator-associated pneumonia, which are frequent risk factors for neonatal sepsis (9). The recent local change in the NICU guidelines for antibiotic therapy has also contributed to a higher incidence of enterobacter infection. In particular, the use of third-generation cephalosporin can have a significant impact on selection of resistant Enterobacterales with the chromosomal AmpC overproduction phenotype (10). The review of protocols with antibiotic referring pediatricians is as important as hygiene measures, to prevent another outbreak with multi-drug resistant bacteria.

In conclusion, reinforcement of standard and contact precautions were the first measures implemented. Disinfection of breast pumps by mothers was established by the infection

control team. Collective discussion with medical and paramedical staff is essential to understand care issues in order to adapt the recommendations. Reducing the empirical use of broad-spectrum antibiotics in NICU is still difficult and has to be discussed collectively with antibiotic prescribers. A multidisciplinary approach remains essential to successfully control an outbreak and prevent the emergence of further outbreaks.

Figure 1. Pulsed-field gel electrophoresis of 11 *Enterobacter cloacae complex* isolates from eight neonates during the outbreak period. Case's number are indicated in front of each line, with the origin and the antibiotic pattern.

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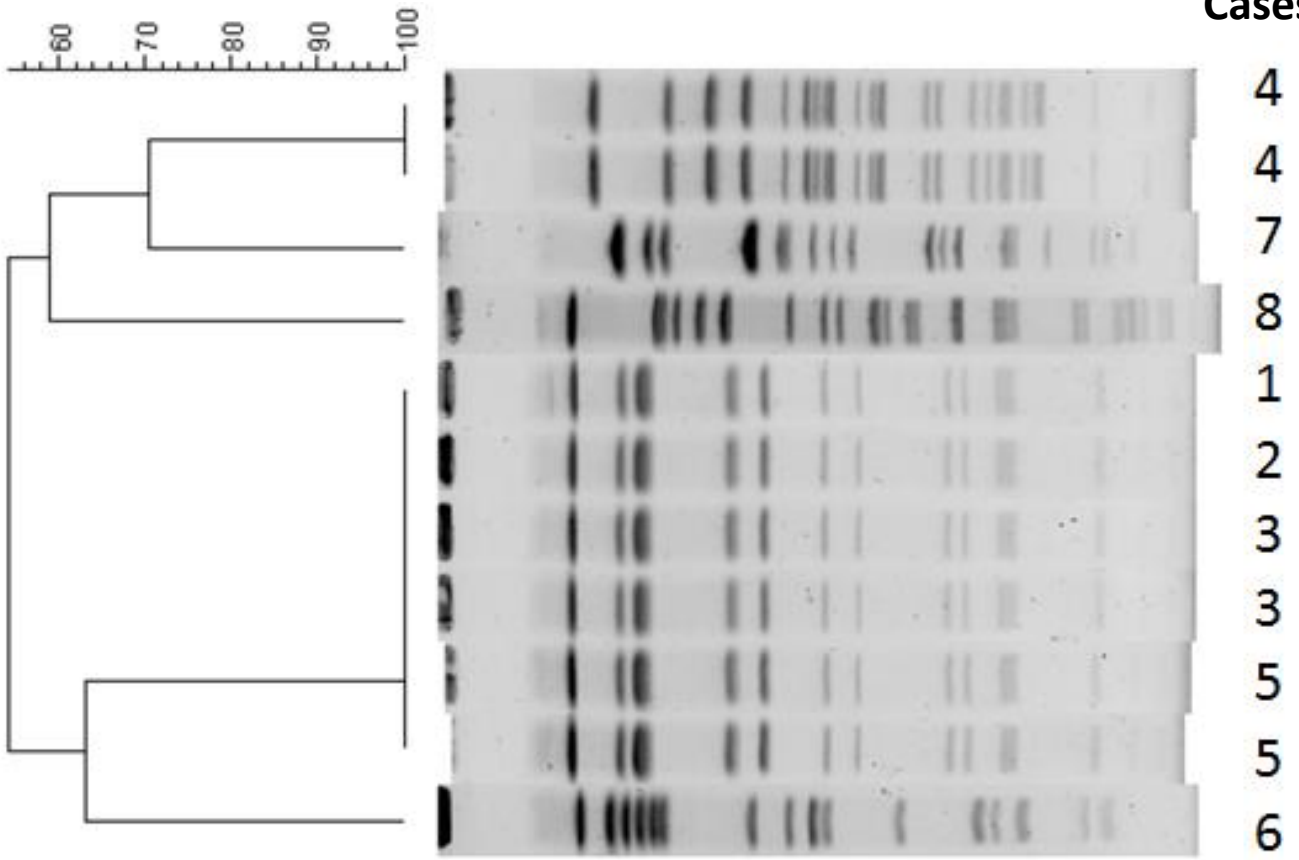
Figure 1. Pulsed-field gel electrophoresis of 11 *Enterobacter cloacae complex* isolates from eight neonates during the outbreak period Case's number are indicated in front of each line, with the origin and the antibiotic pattern



Cases	Samples	Antibiotic pattern
4	Blood	Extended spectrum β-lactamase producer
4	Urine	Extended spectrum β-lactamase producer
7	Blood	Wild type
8	Stool	Wild type
1	Blood	Chromosomal AmpC overproducer
2	Blood	Chromosomal AmpC over producer
3	Blood	Wild type
3	Blood	Chromosomal AmpC overproducer
5	Blood	Chromosomal AmpC overproducer
5	Trachea	Chromosomal AmpC overproducer
6	Skin	Wild type

Table 1. Description of neonatal and maternal risk factors and *Enterobacter cloacae* complex isolates from neonates in an outbreak context

Case	Term	Maternal factors and perpartum antibiotics	Invasive Procedures	Neonatal morbidity	Delay and Date of positive sample	Bacteriological samples
1	Twins preterm (24 weeks GA+4days)	Premature rupture of membranes. Amoxicillin	Intubation Percutaneous catheter	Necrotizing Enterocolitis (grade III) with digestive perforation	Day 4 (09/11/2020)	Blood culture: *E. cloacae complex* TGCR
2			Intubation Umbilical CVC Percutaneous catheter Nasogastric tube Parenteral nutrition	Ventilator-associated pneumonia	Day 11 (16/11/2020)	Trachea sample: *E. cloacae phenotype* and *E. faecalis* Blood cultures: *Staphylococcus haemolyticus*, *S. capitis* and *S. epidermidis*
3	Preterm (26 weeks GA +3days)	Prolonged rupture of membranes (>18 hours) Vaginal swab with *Gardnerella vaginalis* Chorioamnionitis with	Umbilical CVC Percutaneous catheter	Sepsis after catheter removal	Day 9 (16/11/2020)	Blood culture (at birth): *E. coli* Blood culture: *E. cloacae* wild-type and TGCR

		Escherichia coli Amoxicillin				
4	Preterm (24 weeks GA +6days)	Prolonged rupture of membranes (>18 hours) Amoxicillin	Intubation Umbilical CVC Epicutaneous catheter Parenteral nutrition	Necrotizing Enterocolitis (grade II)	Day 7 (27/11/2020)	Blood culture: *E. cloacae* complex ESBL Trachea sample: *E. cloacae* complex ESBL Urinalysis: *E. cloacae* complex ESBL (colonization)
5	Preterm (26 weeks GA)		Intubation Parenteral nutrition Umbilical CVC Percutaneous catheter	Ventilator-associated pneumonia	Day 12 (06/12/2020)	Blood culture: *E. cloacae complex* TGCR Trachea sample: *E. cloacae complex* TGCR Urinalysis: *E. cloacae complex* TGCR
6	Preterm (29 weeks GA)	Prolonged rupture of membranes (>18 hours) Vaginal swab with	Parenteral nutrition Nasogastric tube Umbilical CVC		Day 14 (15/01/2021)	Skin sample: susceptible *E. cloacae* complex

		Haemophilus influenzae Chrorioamnionitis (maternal fever with antibiotic therapy) Amoxicillin + gentamicin	Peripheral venous catheter			
7	Full term (39 weeks GA +1day)	Vaginal swab with Group B streptococcus Penicillin G	Oesophageal atresia Intubation Internal jugular CVC	Ventilator-associated pneumonia	Day 31 (24/01/2021)	Blood culture: susceptible *E. cloacae* complex
8	Full term (37 weeks GA+2 days)	Vaginal swab with Group B streptococcus	Intubation Nasogastric tube	Necrotizing Enterocolitis (grade II)	Day 25 (22/12/2020)	Stool sample: susceptible *E. cloacae complex* (stool colonization)

CVC: central veinous catheter ; TGCR: third-generation cephalosporin resistance ; ESBL : high level β-lactamase production ; ; NEC : Necrotising Enterocolitis according to Bell classification