American Journal of Infection Control 42 (2014) 456-8

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Brief report

Outbreak of AmpC β-lactamase-hyper-producing *Enterobacter cloacae* in a neonatal intensive care unit in a French teaching hospital

Nathalie Pestourie PharmD [a,b,c], Fabien Garnier PharmD, PhD [a,b,c], Olivier Barraud PharmD, PhD [a,b,c], Antoine Bedu MD [d], Marie-Cécile Ploy PharmD, PhD [a,b,c,*], Marcelle Mounier PharmD, PhD [a,b,c]

[a] *INSERM, U1092, Limoges, France*
[b] *Université de Limoges, UMR-S1092, Faculté de Médecine, Limoges, France*
[c] *Laboratoire de Bactériologie-Virologie-Hygiène, CHU Limoges, Limoges, France*
[d] *Service de Réanimation Nénonatale, CHU Limoges, Limoges, France*

Key Words:
Cross transmission
Breast-feeding
Pulsed-field electrophoresis

We report the investigation of an outbreak of colonization by a wild-type *Enterobacter cloacae* and its AmpC-hyper-producing derivative *E cloacae* in a neonatal intensive care unit. *E cloacae* hyper-producing AmpC β-lactamase isolates were found from neonate specimens and from environmental samples. All the isolates belonged to the same clone.

Enterobacter cloacae is a human gut commensal bacteria. It rarely causes infections in the community but is an emerging opportunistic pathogen in the hospital setting,[1] particularly in neonatal intensive care units (NICU).[2,3] Cross transmission via health care workers appears to be a more important factor than environmental contamination.[4,5] We describe here an outbreak with 4 cases of neonate colonization by an *E cloacae* clone hyper-producing the chromosomal AmpC β-lactamase (ECHAC).

METHODS

Case descriptions

The outbreak began in October 2008 when ECHAC isolates were recovered from three 3-week-old babies: twin females (case 1 and case 2: from endotracheal aspirate and rectal swab, each) and a male (case 3: from rectal swab). A wild-type *E cloacae* strain was also isolated from case 2. Three weeks later, ECHAC was recovered from a highly preterm female (case 4: from endotracheal aspirate, rectal swab, and catheter) (Fig 1). All the babies were simply colonized; none developed *E cloacae* infection.

Outbreak investigation

An epidemiologic investigation was begun, and the hospital history of neonates was reviewed. All the neonates hospitalized in the NICU were screened by rectal swabbing, and the infection control team visited the NICU twice to evaluate practices and to collect environmental samples.

Microbiologic analyses

Bacterial isolates were identified with the Vitek2 system (bioMérieux, Marcy-l'Étoile, France), and antibiotic susceptibility was determined with the disk diffusion method, according to the Antibiogram Committee of the French Society for Microbiology (www.sfm-microbiologie.org). The status of AmpC hyper-producing strain was determined phenotypically and was not confirmed by polymerase chain reaction. The different *E cloacae* isolates were compared by means of pulsed-field gel electrophoresis (PFGE) after *Sma*I digestion, using the Gene Path system (Bio-Rad, Hercules, CA).

RESULTS

Cases

No other *E cloacae* isolated in the hospital were related to the NICU cases between October 2007 and October 2008. All 4

* Address correspondence to Marie-Cécile Ploy, PharmD, PhD, CHU de Limoges, Laboratoire de Bactériologie-Virologie-Hygiène, 2 av ML King, 87042 Limoges, France.
E-mail address: marie-cecile.ploy@unilim.fr (M.-C. Ploy).
Conflicts of interest: None to report.

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http://dx.doi.org/10.1016/j.ajic.2013.11.005

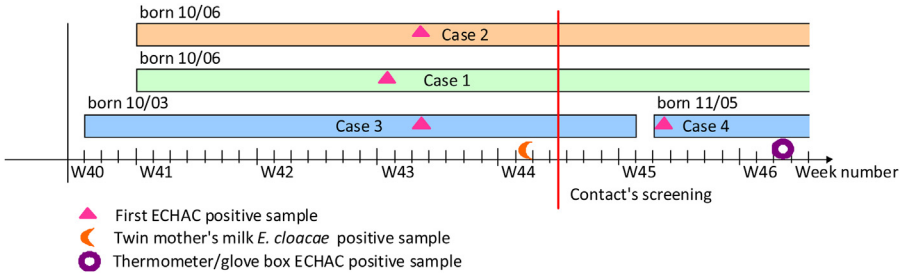

Fig 1. Chronology of the outbreak.

colonized neonates were preterm, and all were born by cesarean section in a dedicated surgical room. They had all been transferred to the NICU immediately after birth. The first 3 neonates were hospitalized in adjacent rooms, and case 4 was hospitalized in the same room of case 3 (Fig 1). In keeping with the unit's standard protocol, all the preterm neonates received antibiotics (amoxicillin, cefotaxime, and gentamicin) at birth.

In the first 3 cases, all rectal samples collected at birth were negative for *E cloacae*, and ECHAC was isolated after more than 2 weeks of hospitalization. A wild-type *E cloacae* strain was also isolated from case 2. In case 4, the ECHAC strain was first isolated at birth from the rectal swab.

Cases 1 and 2 (twins) were fed with their mother's milk, which was expressed directly into feeding bottles at her home. The other case patients were fed with donor milk bank prepared in the NICU. The twins' mother's milk complied with French official standards ($<10^6$ colony-forming units/mL for the viable aerobic flora and $<10^4$ colony-forming units/mL for *Staphylococcus aureus*), but culture of a milk sample yielded rare colonies of wild-type *E cloacae*. The twins' mother's vaginal sample obtained at delivery was negative for *E cloacae*.

Among the 11 environmental samples (surfaces and medical devices), performed after the case 4 detection, ECHAC was isolated from a rectal thermometer and from a glove box in the room where case 4 was hospitalized after case 3.

Screening of all the neonates hospitalized in the NICU identified another baby carrying an ECHAC isolate (screening case). All her previous samples had been negative for *E cloacae*.

PFGE showed that all the isolates, including the wild-type milk isolate and the 2 environmental ECHAC isolates, belonged to the same clone, with an exception for the screening case (Fig 2).

Control measures

Hygiene rules and care procedures were reinforced, and the premises were thoroughly cleaned. Some practices were reviewed such as changing diaper, which was performed without gloves. During the investigation, we noted minor protocol violations, in cleansing of the thermometer, which was realized by simple wiping without immersion in a detergent-disinfectant solution. The staff was reminded of the protocol, and its correct implementation was audited.

Systematic ECHAC surveillance was continued on all clinical samples collected in the NICU for 2 months. No other ECHAC isolates were recovered during this period.

DISCUSSION

A total of 12 *E cloacae* isolates (wild-type and resistant) with the same PFGE profile was recovered from the 4 babies, maternal milk, and environmental samples during the outbreak period. Because the wild-type isolate recovered from maternal milk belonged to the same clone as the resistant isolates, we suspected that the clone was transmitted to the twins via their mother's breast milk. Breast milk has been previously shown to be capable of transmitting viruses[6] and bacteria such as *Enterobacter aerogenes*.[7]

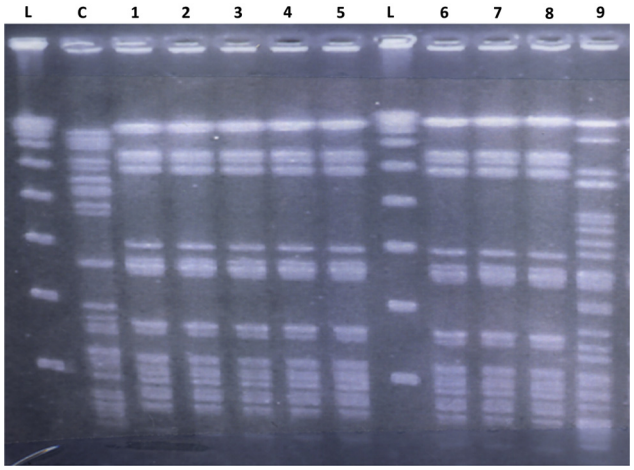

Fig 2. PFGE profiles of the *E cloacae* strains. L, ladder; C, control; 1, case 1 (ECHAC); 2, case 2 (ECHAC); 3, case 2 (wild-type); 4, case 3 (ECHAC); 5, case 4 (ECHAC); 6, mother milk (wild-type); 7, glove box (ECHAC); 8, thermometer (ECHAC); 9, screening case (ECHAC).

Theoretically, the clone could also have spread via the milk preparation, but this possibility was ruled out because the only positive milk sample came from the twins' mother. A contact transmission between the twins' mother and her babies could not be excluded.

We suspected that the clone initially had a wild-type phenotype and that a mutant hyper-producing the AmpC β-lactamase was selected by antibiotic pressure in the twins. The ECHAC strain may have been transferred to case 3 via health care workers' hands or environmental contamination.

Case 4 occurred after reinforced control measures had been implemented. It involved a highly preterm female born by emergency cesarean section and transferred immediately to the NICU, in the room previously occupied by case 3. Among the environmental samples, an ECHAC isolate was recovered from the rectal thermometer used for case 4. Discussion with the staff identified a reversal of normal procedures because of the emergency delivery, with the temperature being taken just before rectal swabbing for bacteriologic analysis. The thermometer therefore appears to have been the source of colonization in case 4, although we cannot rule out transmission via the nursing staff because ECHAC was also found on a glove box in the same room. The potential role of thermometers in bacterial transmission has previously been described.[8,9]

We describe here an outbreak of colonization by a multidrug-resistant strain in a NICU. The fact that all the isolates belonged to the same clone points to the following chronology: introduction