Molecular Characterization of a Rare ESBL *E. coli* Strain Causing a Necrotizing Enterocolitis Outbreak in Preterm Infants

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Background: Necrotizing enterocolitis (NEC), a severe comorbidity of prematurity, is usually sporadic, but occasional outbreaks suggest an infectious cause. *Escherichia coli*, the most frequent Gram-negative pathogen in preterm infants, historically displays a low inhospital transmissibility.

Aim: To report the management of an NEC outbreak in a Belgian neonatal intensive care unit and the molecular characterization of a rare, highly virulent/resistant *E. coli* strain.

Methods: Clinical data were extracted from electronic medical records. Surveillance and clinical isolates characterized using standard methods were secondarily analyzed by bacterial whole-genome sequencing using EnteroBase for phylogenic classification and BioNumerics for resistance and virulence profile determination.

Findings: A cluster of 6 infants was colonized by a single extended-spectrum beta-lactamase-producing *E. coli* strain in a 1-month period. Four infants developed severe NEC, resulting in 1 death and 3 short bowel syndromes. Although the index infant and his twin sibling acquired the strain vertically from their mother, transmission occurred horizontally through caregivers in subsequent cases. Enhanced infection prevention and control measures allowed containment of the outbreak. Molecular typing of the strain revealed a single, previously unregistered O6:H1 serotype of extraintestinal pathogenic *E. coli*, urinary pathogenic *E. coli* harboring multiple resistance and virulence genes, including extended-spectrum beta-lactamase-encoding $bla_{CTX\text{-}M\text{-}15}$ and fimbriae-encoding *papA*.

Conclusion: The emergence of high-virulence strains in neonatal intensive care units calls for the implementation of enhanced infection prevention and control strategies. Bacterial genomic sequencing techniques, if implemented in multidrug-resistant organism screening, could represent a valuable addition for early characterization of virulence and resistance profiles, and improve prevention and containment of infectious outbreaks.

Key Words: *Escherichia coli*, infectious disease outbreak, neonatal intensive care unit, necrotizing enterocolitis, bacterial genome

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Necrotizing enterocolitis (NEC), one of the most prevalent and severe gastrointestinal comorbidities in neonates, has a worldwide incidence estimated at 7% in infants <32-week gestational age with wide variations according to environment, populations and sociodemographics.[1] If low birth weight and gestational age represent the main risk factors, NEC occurs within a spectrum of gastrointestinal disorders, including dysmotility, immature barrier function,[2] dysbiosis,[3] decreased mesenteric arterial perfusion,[4] microvasculature maldevelopment[5] or immature innate immunity with deficient regulatory mechanisms.[6] These disorders may coexist and trigger a common pathway of bowel wall distension, ischemia, transmucosal bacterial invasion, uncontrolled inflammation and, ultimately, bowel necrosis when untreated.[7,8]

Various intrinsic and environmental factors predispose premature infants to NEC, such as excessive toll-like receptor 4 signalling[9] resulting in uncontrolled inflammatory responses to lipopolysaccharide, broad-spectrum antibiotics exposure leading to dysbiosis,[10–16] a lack of reliable predictive biomarkers,[17,18] or higher formula feeding exposure due to insufficient maternal breastmilk and limited access to human milk banks.[19]

Although NEC is typically sporadic, temporal clusters occur,[20–23] suggesting that specific pathogens, mostly bacteria but also fungi and viruses, play a relevant role.[24] Enterobacterales, more specifically *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*, are the most frequent pathogens associated with NEC.[25] While enterobacterales are commonly isolated from intra-abdominal samples of infants with NEC, blood cultures are often negative,[26] suggesting that infection originates primarily from the gut, following prior intestinal colonization.[27]

Following an episode of lethal NEC associated with an extended-spectrum beta-lactamase producing (ESBL) *E. coli*, we observed a rapid spread of the strain across the neonatal intensive care unit (NICU), leading to an NEC cluster. We report the molecular characterization of this strain, which revealed a unique combination of resistance and virulence factors, and describe the infection prevention and control (IPC) measures allowing its containment.

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All data are accessible from the corresponding author upon reasonable request.

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METHODS

Demographics, clinical and microbiological data were collected retrospectively from electronic medical records. NEC onset was defined using the date of diagnosis. Based on IPC data, we calculated ESBL *E. coli* acquisition density incidences, defined as the number of acquisitions/1000 patient days for the outbreak month and the 12-month periods preceding and following it.

For multidrug-resistant organisms (MDRO) surveillance, nose, throat, skin and rectum swabs are systematically collected 4 days after birth in inborn infants or upon admission in outborn, then weekly until discharge. Screened MDRO include ESBL-producing Enterobacterales, carbapenemase-producing Enterobacterales, carbapenemase-producing Gram-negative nonfermenters, vancomycin-resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus*. Detection of an MDRO strain prompts contact precautions and single room isolation, except for ESBL *E. coli*. For infants <28 weeks gestational age and/or <1.0 kg, maternal breastmilk is cultured at 72 hours of life, then weekly until 28 weeks corrected age. Maternal breastmilk is administered raw if total bacterial count is ≤10,000 colony-forming units (CFU) per mL, administered after pasteurization if >10,000 to ≤100,000 CFU/mL, and discarded if >100,000 CFU/mL. For Gram-negative bacteria (GNB), breastmilk is administered raw if ≤100 CFU/mL are detected, pasteurized for >100 to ≤10,000 CFU/mL and discarded for >10,000 CFU/mL. Antibiotic resistance profiling is not routinely performed for breastmilk isolates.

MDRO surveillance and clinical samples were cultured on standard selective media. Clinically indicated bacteriological analyses included blood, urine and peritoneal/intestinal samples collected during surgery [peroperative digestive samples (PODS)]. Strain identification was carried out by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Microflex, Bruker Daltoncis, Bremen, Germany). Automated antimicrobial susceptibility tests (Phoenix, Becton Dickinson, NJ) were interpreted according to European Committee on Antimicrobial Susceptibility Testing 2019 antimicrobial breakpoints (https://www.eucast.org). ESBL phenotype was determined according to the European Committee on Antimicrobial Susceptibility Testing algorithm for detection of resistance mechanisms v 2.0.

Bacterial genome sequencing was performed on a NovaSeq 6000 instrument (Illumina, San Diego, CA).[28] The raw reads were uploaded and *de novo* assembled using SPAdes v.3.7.1 in BioNumerics v.8.1.1 (Applied Maths, BioMérieux, Sint-Martens-Latem, Belgium). The *E. coli* genotyping tool v.2.1 was used to predict *E. coli* pathotypes, serotypes, virulence gene profiles, acquired resistance genes and point mutations, with minimum identity and coverage thresholds were set at 85% and 60%, respectively. The raw reads were also uploaded and automatically assembled in the public genome database EnteroBase.[29] Sequence types (STs) were determined using the Achtman seven-gene multilocus sequence typing (MLST) scheme. Clustering and cluster affiliation were performed by core genome MLST analysis. The cluster threshold was set at hierarchical clustering (HC) level HC5, that is, all isolate genomes in this cluster have links ≤5 alleles apart. A GrapeTree minimum spanning tree based on the EnteroBase core genome MLST was generated using the MSTree V2 algorithm.[30] All genomes are available online in the EnteroBase Escherichia/Shigella database (https://enterobase.warwick.ac.uk) [ESC_CB5101AA to ESC_CB5104AA; ESC_KB9761AA].

RESULTS

This NEC cluster occurred between September 12 and October 11, 2022, in a 29-bed level-IV NICU, constituted of 6 multibed rooms with a 16-patient capacity (floor 1), and 9 single-family rooms with a 13-patient capacity (floor 2), staffed with 7 neonatologists, 6 residents and 80 nurses.

Patients' baseline characteristics, microbiological data, NEC management and outcomes are summarized in Table 1. The index case (no. #1) was a 24-week preterm twin delivered by cesarean section because of preterm premature rupture of membranes (PPROM) and chorioamnionitis. The infant received amoxicillin and amikacin for rule-out sepsis at birth, but antibiotics were discontinued after 48 hours of life as blood culture were negative. This infant was advancing on enteral feedings (up to 90% with 20 mL/kg daily increases per protocol) with formula, as mother's milk resulted contaminated with *E. coli* and *Klebsiella pneumoniae*. He presented with fulminant NEC and shock at 12 days of life. Near-total intestinal necrosis was found at laparotomy (Figure, Supplemental Digital Content 1, https://links.lww.com/INF/G258), and the infant was redirected to comfort care. During the following weeks, 3 other infants (nos. #2, 3 and 4) presented with fulminant, modified Bell stage IIIb NEC (Table, Supplemental Digital Content 2, https://links.lww.com/INF/G259).[31] Patient no. #2, the twin sibling of no. #1, was on full formula feedings, while the other 2 were fed with mother's milk. In all cases, ischemic and necrotic lesions involving large areas of the small intestine and colon were present at laparotomy. The 3 survivors underwent multiple surgical revisions with segmental resections, developed short gut syndrome and required prolonged antibiotic therapy and parenteral nutrition. One of these infants subsequently died out of a central line placement complication. In all 4 infants, a phenotypically identical ESBL *E. coli* strain was found on PODS. Blood cultures sampled before antibiotics initiation were negative.

The timeline of ESBL *E. coli* acquisition and NEC episodes is summarized in Figure 1. The strain was detected for the first time on patient no. #2 in a rectal surveillance culture on the fifth day of life. In his twin sibling (no. #1), day-of-life 5 surveillance cultures were missing; those performed on day-of-life 12 resulted negative. However, when this infant presented with NEC and underwent surgery on day-of-life 13, PODS cultures resulted positive for the strain. On the following week, 2 other infants (nos. #5 and 6) tested positive for the strain, but remained asymptomatic carriers throughout hospitalization. Two more infants (nos. #3 and 4) acquired the strain after 2 weeks, and developed NEC. No other horizontal contamination was detected thereafter. All infants continued to carry the strain until discharge or demise.

In retrospect, we could trace the origin of the outbreak to the mother of nos. #1 and 2, a 31-year-old gravida-2 para-0 immunocompetent woman who was hospitalized in the maternal intensive care unit for a cervical cerclage at 18 6/7 weeks. Although she had received prophylactic cefuroxime, she developed an ESBL *E. coli* urinary tract infection 3 days after the procedure, and was treated with amoxicillin-clavulanate for a total of 14 days. She was rehospitalized at 23 3/7 weeks for PPROM, developed chorioamnionitis and was treated with cefuroxime, as the amniotic fluid and vaginal cultures only showed a mixed anaerobe flora and *Candida albicans*, respectively. The ESBL *E. coli* strain was not detected at that time. The babies were delivered by C-section at 24 1/7 weeks and hospitalized in the NICU. Mother's milk screening cultures showed >10.000 Gram-negative CFU/mL (*E. coli* and *K. pneumoniae)*; thus, the infants were fed with preterm formula, as no donor milk was available at our hospital at that time. Hence, the strain was likely transmitted vertically around delivery, or by skin contact in the following days. Following this mother-to-infant transmission, the strain spread horizontally in the NICU presumably through nurses who oversaw 2–3 patients per shift located in different rooms (Figure, Supplemental Digital Content 3, https://links.lww.com/INF/G260), although we cannot exclude medical staff involvement in transmission. When patients nos. #5 and 6 resulted contaminated

TABLE 1. Clinical Profiles and Outcomes

	Patient 1 (Index) (Twin A)	Patient 2 (Twin B)	Patient 3	Patient 4	Patient 5	Patient 6
Date of birth	08/30/2022	08/30/2022	09/20/2022	09/01/2022	07/26/2022	09/12/2022
	Male	Female	Male	Male	Female	Male
Gestational age	24 weeks 1 day	24 weeks 1 day	29 weeks 3 days	34 weeks 6 days	24 weeks	23 weeks 6 days
Birthweight (Z score)	590 g (−0.5)	660 g (+0.37)	1,115 g (−0.4)	1,440 g (−2.3 (SGA)	565 g (−0.32)	685 g (+0.37)
Delivery mode	C-section	C-section	C-section	Vaginal	Vaginal	Vaginal
Date/age at strain detection	Undetected	09/05/2022 5 days	09/26/2022 5 days	09/26/2022 24 days	09/19/2022 55 days	09/19/2022 6 days
Date/age at NEC diagnosis	09/12/2022 12 days	10/11/2022 41 days	09/28/2022 9 days	09/25/2022 24 days	No NEC	No NEC
Modified Bell's staging	Stage IIIb	Stage IIIb	Stage IIIb	Stage IIIb	n/a	n/a
Feeding regimen at NEC onset or strain detection	90% enteral, preterm formula	100% enteral, preterm formula	57% enteral, fortified MBM	100% enteral, fortified MBM	100% enteral, fortified MBM	25% enteral, MBM
Prior bacterial infections	Day 0: Presumed EOS	Day 0: presumed EOS Day 16: non-ESBL *E. coli* UTI and *KLP* bacteremia	Day 1: culture-negative EOS	Day 2: non-ESBL *E. coli* meningitis	Day 0: *KPL* EOS Day 30: *KLP* LOS	Day 0: culture-negative EOS
Prior antimicrobial exposure	AMO + AMI (2 days) FLZ (12 days)	AMO + AMI (2 days CTA + VAN (7 days) MER (21 days) FLZ (40 days)	AMO + AMI (7 days)	AMO (23 days) AMI (2 days) CTA (16 days)	AMO + AMI (3 days) CTA (25 days) VAN (7 days) FLN (7 days)	AMO + AMI (2 days) CTA + VAN (5 days)
NEC medical management	HFOV Vasopressors PIT (1 day)	IPPV (18 days) Vasopressors (13 days) MER (26 days) VAN (21 days)	IPPV (7 days) Vasopressors (2 days) PIT (17 days)	IPPV (22 days) CTZ + MET (4 days) PIT (14 days) MER (18 days)	n/a	n/a
NEC surgical management	Day 12: laparotomy, redirection of care	Day 41: ileostomy Day 43: ileal resection, jejunostomy Day 123: ICV resection, restoration of continuity	Day 10: ileal resection, jejunostomy Day 72: ICV resection, restoration of continuity	Day 25: ileal resection, jejunostomy Day 32: ileal resection Day 99: distal colon resection, restoration of continuity	n/a	n/a
Residual bowel	n/a	Small intestine 20 cm + colon	Small intestine 25 cm + colon	Small intestine 35 cm + partial colon	n/a	n/a
Hospitalization outcome	Death, redirection of care (day 13)	Death, CVC complication (day 115)	Discharged, 37% home TPN (day 188)	Discharged, 45% home TPN (day 190)	Discharged, full oral feeds (day 107)	Discharged, full oral feeds (day 100)

AMI indicates amikacin; AMO, amoxicillin; CTA, cefotaxime; CTZ, ceftazidime; CVC, central venous catheter; EOS, early-onset sepsis; FLN, fluconazole; HFOV, high-frequency oscillatory ventilation; ICV, ileo-cæcal valve; IPPV, Intermittent positive pressure ventilation; IVH, intraventricular hemorrhage; *KLP*, *Klebsiella pneumoniae*; LOS, late-onset sepsis; MBM, maternal breast milk; MER, meropenem; MET, metronidazole; NEC, necrotizing enterocolitis; PIT, piperacillin-tazobactam; SBS, short bowel syndrome; SGA, small for gestational age; TPN, total parenteral nutrition; UTI, urinary tract infection; VAN, vancomycin.

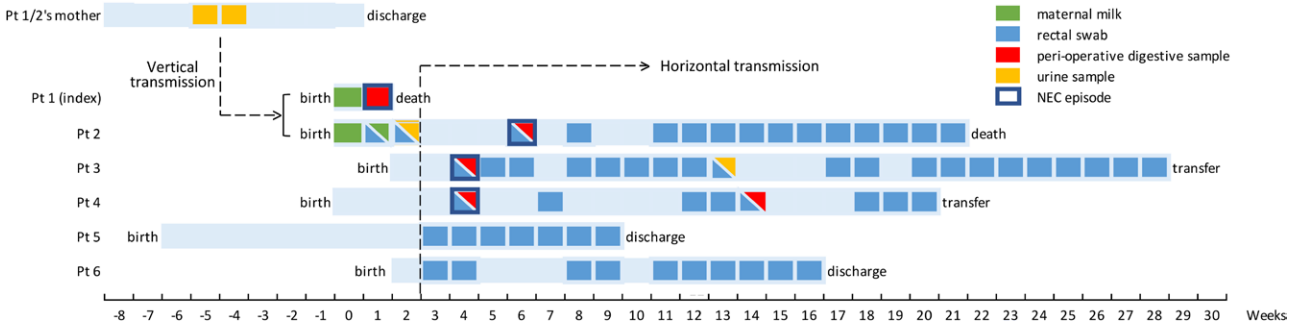

FIGURE 1. Timeline of bacterial isolates positive for ESBL *E. coli* in contaminated and infected subjects.

on September 19, 2022, surveillance rectal swabs andcontact precautions (single-use hats, gloves and gowns) were implemented. In addition, the hospital IPC team provided all NICU caregivers with enhanced hand-hygiene education. When further spread was detected in infants nos. #3 and #4 on September 26, 2022, carriers were either cohorted in multirooms or isolated in single rooms, with dedicated nursing staff. ESBL *E. coli* acquisition density incidence peaked at 12.4/1000 patient-days during the month of September 2022, while values were 0.14 and 1.7/1000 patient-days during the preceding and following 12-month periods, suggesting this outbreak was a sporadic event largely determined by the strain's resistance and virulence.

Clinical and surveillance isolates displayed an identical antimicrobial resistance phenotype, including resistance to third- and fourth-generation cephalosporins and aztreonam, with susceptibility to amoxicillin-clavulanic acid and piperacillin-tazobactam, whole-genome sequencing (WGS) analysis of PODS isolates from the 4 NEC subjects and urine isolates from nos. #1 and 2's mother demonstrated an identical strain, characterized as extraintestinal pathogenic *E. coli* (ExPEC), Uropathogenic *E. coli* (UPEC), O6:H1 serotype, ST73 (B2 phylogroup). All isolates carried the same repertoire of putative virulence genes involved in adhesion, invasion, iron uptake, cell toxicity and evasion of the host immune response (Table 2). In addition, all isolates harbored the $bla_{\text{CTX-M-15}}$ ESBL gene, conferring resistance to most cephalosporins and monobactams, and the *gyrA* (Ser83Leu) mutation linked to fluoroquinolone resistance in *E. coli*. The genome isolates, ST 73, were closely related (allelic difference range: 0–1) and belonged to cluster HC5_220417. This cluster appears to be unique, as no other isolate genomes in Enterobase have links ≤10 alleles apart (Figure, Supplemental Digital Content 4, https://links.lww.com/INF/G261).

DISCUSSION

From the early 2000s, following the wide implementation of maternal group-B Streptococcus prophylaxis, a reduced incidence of early-onset Gram-positive sepsis was mirrored by an increase in infections with more virulent Gram-negative organisms.[32] *E. coli* has since become the most prevalent pathogen in neonatal early-onset sepsis, especially in preterm infants.[33] Studies conducted in low-income countries with a high infant sepsis burden underscore a key role of mother-to-infant transmission in neonatal GNB colonization, infection and mortality.[34,35] In a large cohort of low-income mother-infant dyads, maternal ESBL GNB carriage resulted an independent risk factor of neonatal sepsis and adverse birth outcome, with a high prevalence of identical strains between mothers and their infants.[36] Similarly, a large WGS-based study conducted in 2 European NICUs showed that maternal carriage was the main independent risk factor for ESBL GNB contaminations in very low birth weight infants.[37] These studies suggest that systematic screening of both neonates and mothers could contribute to improve neonatal outcomes in high-risk pregnancies.

The intestinal microbiome of premature infants is characterized by low levels of anaerobic bacteria and high levels of potentially pathogenic bacteria such as Enterobacterales.[8,15,38] A shift of the microbiome toward Proteobacteria species, especially pathogenic Enterobacterales, represents a major risk factor for NEC.[16,39–44] The intestinal microbiome is shaped at birth by maternal vaginal, rectal and cutaneous microbiota. Babies delivered via C-section tend to be colonized by a flora resembling mother's skin, while those born vaginally acquire a vaginal flora type.[9] Microbiota acquisition may start antenatally through fetal ingestion of amniotic fluid.[45] A meta-analysis of 55 studies demonstrated the association between chorioamnionitis and neonatal sepsis, with a 3–6-fold increased risk in preterm infants.[46] Although we failed to isolate the causative ESBL *E. coli* strain in the mother's amniotic fluid or placenta, it is possible that the fetus intestine was colonized in utero following PPROM. We cannot exclude that the infants received small amounts of mother's milk before it was discarded for contamination. Alternatively, the infants may have acquired the strain through postnatal skin-to-skin contact. A prospective study in extremely premature babies showed a correlation between late-onset sepsis and initial breastmilk contamination with *E. coli*, not observed with other pathogens.[47] In our NICU, all infants <32 weeks receive probiotics from enteral feeding onset. Probiotics

TABLE 2. Whole-genome Sequencing (WGS)-based Virulence-associated Gene Profiles and Antimicrobial Resistance Profiles for Peroperative Digestive Samples (Infants) and Urine (Mother) *E. coli* Isolates

		Virulence Factors								Resistance Factors	
Patient	Isolate	Adherence	Invasion	Iron uptake	Proteases	Regulation	Secretion	Stress	Survival	Toxin	Gene	Variant
1	16257	*Hra*	*ompT*	*ireA*	*Gad*	*terC*	*kpsMII_K5*	*usp*	*iss*	*Clb*	$bla_{\text{CTX-M-15}}$	*gyrA*(Ser83Leu)
2	16313	*iha*		*sitA*	*Pic*		*kpsE*			*cnf*		
3	16316	*irp2 focCsfuE papA_fteA_F10*		*chuA*	*Sat*					*hlyA*		
4	16318	*papC papA_F19*		*fyuA*	*vat*					*tcpC*		
Mother 1 and 2	16108	*sfaD, yfcV*		*iucC*								
				iutA								

reduce NEC incidence[7,48,49] by competing with pathogenic microbiota and modulating innate immunity.[50] As NEC declared early in some of our cases, we may speculate that probiotic exposure was insufficient to balance the strain's intestinal proliferation.

After the index case's demise and the sibling's contamination, horizontal contamination was detected in 4 nonrelative infants in the following week. No new acquisitions occurred after enhanced IPC measures were implemented. However, ESBL *E. coli* acquisition density in the 12 months following the outbreak remained slightly higher than in the preceding 12 months, although the strains involved were phenotypically different from the outbreak strain. Cross-sectional studies of intestinal microbiota among hospitalized neonates show diversity of GNB strains evolving over time with no evidence of clonality, suggesting continuous environmental exposures.[35] Although such a mechanism does not apply to the current outbreak, it highlights the importance of strict IPC surveillance in NICUs. Specific guidelines on MDRO screening and IPC measures in neonatology are scarce, and best practices, benefits and potential harms are still controversial, as underlined in the European standards of care for newborn health recommendations on patient screening for resistant bacteria (https://newborn-health-standards.org). A single-NICU Swedish study has shown that a systematic screening policy, instead of elective screening when ESBL GNB infections occur, resulted in an 8-day reduction in admission-to-detection time and a drastic reduction in secondary acquisitions and infections.[51] Unlike for most MDRO, universal guidelines do not recommend enhanced IPC measures such as isolation or contact precaution for ESBL *E. coli*, because person-to-person transmission is considered as uncommon in hospital settings.[52,53] In our NICU, enhanced contact precautions and isolation for ESBL *E. coli* carriage are implemented once an acute rise in incidence density is detected. In this outbreak, the IPC response was triggered by the unusually early and severe clinical presentation of the index patient and the rapid spread across the unit, suggesting a highly virulent and transmissible strain. Following this outbreak, we improved electronic medical record flagging of MDRO detected in the maternal intensive care unit, in order to inform antimicrobial therapies in the NICU. We revised IPC protocols for carrier isolation and cohorting in response to virulent strain detection. We implemented fast and systematic root cause analyses of infection-related undesirable occurrences through an institutional reporting system. We reinforced continuous IPC education among staff, and we are planning for additional single-family rooms, allowing for better infection prevention and containment.

Invasive *E. coli* strains distinct from commensal or enteropathogenic strains, thus termed ExPEC, are a prevalent source of sepsis in neonates.[54,55] However, molecular data on their virulence factors are scarce.[56] In the few studies published to date, most *E. coli* strains involved in NEC belong to the ExPEC/UPEC group and harbor specific STs.[55,56] Colonization by ST73, as in this outbreak, is correlated with NEC and mortality in preterm infants.[55] The zoonotic ST73 UPEC lineage is an emerging cause of urinary tract infection and sepsis in humans,[57] including neonates.[58–60] This strain's genotyping revealed an extended repertoire of adherence factors, including papA uropathogenic fimbriae (P-fimbriae). Type 1 fimbriae and P-fimbriae facilitate adhesion to host enteric epithelial cells, tissue invasion, biofilm formation and cytokine induction. Additional virulence-related genes encoding for flagella, capsular lipopolysaccharides and outer membrane proteins were also present.[61,62] The combination of these virulence factors likely contributed to the strikingly severe bowel injury observed in this outbreak. A large cohort study of adults with *E. coli* sepsis demonstrated that the number of virulence genes harbored correlates with disease severity and clinical outcome.[63]

Over the past 2 decades, the incidence of neonatal GNB infections has dramatically increased, both in high-risk populations from high-income countries[64] and in developing areas of the world,[37] largely because of their increasing resistance to available broad-spectrum antibiotics.[65] This strain's genotyping revealed the presence of the $bla_{CTX\text{-}M15}$ MDR gene. CTX-M proteins predominantly hydrolyze cefotaxime and ceftazidime, with variable activity against aztreonam. CTX-M prevalence is as high as 50% in GNB isolates from neonates with sepsis.[66] CTX-M plasmids frequently carry other resistance genes, resulting in cross-resistance to fluoroquinolones and other antimicrobial classes.[65] This strain harbored the *gyrA* Ser-83 mutation, a recognized cause of quinolone resistance.[67] Interestingly, this strain did not result quinolone-resistant by antimicrobial susceptibility tests, illustrating potential discrepancies between genotype- and phenotype-based approaches.[68] When declaring with NEC, the index patient was empirically treated with piperacillin-tazobactam since the strain had not been isolated before. Subsequent cases were treated based on susceptibility profiles of the surveillance isolates, which included piperacillin-tazobactam and meropenem.

Bacterial genomic analysis is a promising tool both for systematic surveillance and outbreak investigation, as it provides comprehensive data on serotype, pathotype, resistance and virulence factors. A recent study comparing genome-based versus standard techniques for characterization of invasive *E. coli* isolates shows a very high correlation of the antimicrobial resistance profiles.[68] Recent WGS experimental studies[69] and systematic reviews[70] begin to reveal the correlations between single genes and neonatal outcomes, underlining the importance of *E. coli* virulence gene repertoires and the heterogeneity of these genes between strains. Genomic analysis of this strain was conducted in retrospect and did not directly influence outbreak management, which was based on standard clinical and microbiological approaches.

CONCLUSION

This outbreak was caused by a highly resistant, virulent and transmissible ESBL *E. coli* strain originating from a single carrier in the maternal intensive care unit. The strain was transmitted vertically to her twin infants, then spread horizontally to other NICU patients. If validated in prospective studies, systematic molecular characterization of MDRO in maternal and neonatal intensive care settings may emerge as a valuable approach to improve the detection of potentially virulent strains, guide preventive and therapeutic strategies, and pave the way to targeted antivirulence therapies.[71]

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