




Virulence Potential of ESBL-Producing *Escherichia coli* Isolated during the Perinatal Period

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Abstract

Objective The aim of the study was to investigate the virulence factors in *Escherichia coli* producing extended-spectrum β -lactamase (ESBL) derived from the perinatal fecal colonization flora of mothers and their newborns in a Chinese obstetric ward.

Study Design Rectal swabs were obtained from mothers prenatally and from their newborns postnatally, and analyzed for ESBL-producing *Escherichia coli*. The isolates were then whole-genome sequenced.

Results Maternal and neonatal colonization by ESBL-producing *E. coli* in a Chinese obstetric ward was 18% (31/177) and 5% (9/170), respectively. Fecal ESBL-producing isolates exhibited a significantly lower frequency of virulence factors compared with invasive *E. coli*.

Conclusion Providing balanced information on screening results is essential, along with conducting a risk assessment for antibiotic treatment strategies.

Keywords

- ▶ *Escherichia coli*
- ▶ fecal carriage
- ▶ virulence factors
- ▶ extended-spectrum β -lactamase
- ▶ ESBL

Key Points

- High ESBL *E. coli* colonization rates in mothers and neonates perinatally.
- Fecal ESBL-producing *E. coli* showed fewer virulence traits.
- ESBL-producing *E. coli* knowledge may prompt antibiotic overuse.

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Extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* is a recognized public health threat, where it can lead to potentially life-threatening bloodstream infections. *Escherichia coli* bloodstream infections present a substantial challenge within neonatal care and are acknowledged as an important global public health concern.¹ Maternal transmission has been recognized as a significant source of neonatal colonization with *E. coli*, and extended stay in the intensive care unit increases the risk of nosocomial infections due to gram-negative rods such as *E. coli*.² This study aimed to investigate the frequency of ESBL-producing *E. coli* in an obstetric ward in Changchun City, China, a country where a previously reported high prevalence of ESBL-producing Enterobacteriaceae has reached up to 58%.³ The occurrence of virulence factors linked to invasive disease was examined in all ESBL-producing *E. coli* using a recently established virulence database.⁴

Materials and Methods

Patients

Between September 2013 and January 2014, ESBL-producing *E. coli* isolates from fecal samples acquired from pregnant women and their newborns at the Children's Hospital in Changchun City, China, were consecutively collected. The hospital is a referral and teaching hospital in Northeast China, serving approximately 20,000 inpatients annually from a catchment area of approximately 4,050,000 inhabitants. After recording information on whether the isolate originated from a mother or a child, the isolates were treated anonymously. The study was reviewed by the local ethical review board prior to its start, and after obtaining patient consent, fecal samples were collected from both mothers and their newborns. Maternal specimens were obtained through rectal swabs immediately after defecation, while neonatal specimens were collected from feces and transported in charcoal Amies media (Copan, Brescia, Italy) to the laboratory. Maternal specimens were taken upon arrival at the obstetric department before delivery, and one sample was collected from the newborns before they left the maternity ward, approximately 1 week later. Neonates in need of neonatal inpatient care were excluded from the study.

Culture-Based Methods

All swabs were plated on chromogenic urinary tract infection (UTI) agar plates (Oxoid, United Kingdom) and incubated at 35 °C overnight. Subcultivation of presumptive coliform bacteria on chromogenic UTI agar was performed on chromogenic ESBL agar plates (Oxoid, United Kingdom). Presumptive *E. coli* (pink colonies) were susceptibility tested according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org) using a diffusion synergy test with clavulanic acid (10 μ g) and the cephalosporins—cefotaxime (5 μ g), ceftazidime (10 μ g), and cefepime (30 μ g). Isolates were categorized as reduced susceptibility when the zone diameter for cefotaxime, ceftazidime, or cefepime was <17 mm. Isolates were categorized as classical ESBL phenotype when synergy between clavulanic

acid and the tested cephalosporins was observed. For isolates with a negative synergy test but reduced susceptibility to cefotaxime or ceftazidime and cefoxitin (30 μ g; <19 mm), an AmpC-type was suspected. All *E. coli* isolates with suspected ESBL production were stored in glycerol stock at -80°C .

Whole-Genome Sequence Analysis of Bacterial Isolates and Calculations

ESBL-producing *E. coli* isolates were analyzed by whole-genome sequencing. Extraction, library preparation, and bioinformatics analysis were performed as described elsewhere, all sequence data are available through Enterobase (<https://enterobase.warwick.ac.uk/>).⁴ ESBL-production was confirmed by BLASTn (nucleotide Basic Local Alignment Search Tool) searches on draft genomes by using Comprehensive Antibiotic resistance database (CARD) (<https://card.mcmaster.ca/>; October 2020) as reference database. Gene prediction for virulence factors was performed on draft genomes by using the BLASTn algorithm with standard settings and a previously established virulence database for ExPEC (Extraintestinal pathogene *E. coli*).⁴ Virulence factors were interpreted as predicted with a nucleotide coverage of $\geq 99\%$ and nucleotide identity of $\geq 98\%$. Phylotypes were determined according to the Clermont scheme, and the phylogenetic relationship of all isolates was analyzed using hierarchical clustering as implemented in Enterobase (<https://enterobase.warwick.ac.uk/>). The frequency of virulence factors was compared with those from invasive *E. coli* isolates derived from neonates. To do so, a total of 32 isolates published in a previous study, isolated from blood and cerebral spine fluid cultures at Uppsala University Hospital between 2005 and 2015, were previously analyzed accordingly.⁴ Genetic determinants for virulence factors were treated as presence/absence data and statistical computations were performed using the statistical software R (v4.3.0, April 21, 2023, R Foundation for Statistical Computing, Vienna, Austria). Odds ratios and statistical significance were calculated using Fisher's exact test for small sample sizes. All sequence data are publicly available through Enterobase, accession numbers are listed in [Supplementary Material S1](#) (available in the online version).

Results

In the present investigation, a total of 347 samples were analyzed, and ESBL-producing *E. coli* were detected in 18% (31/177) of the maternal samples and 5% (9/170) of the newborns. Hierarchical clustering as implemented in Enterobase defined three isolate pairs, three isolates, and additional four isolates as indistinguishable, suggesting maternal or health care-related transmission to the newborn. All phylogenetic lineages according to Clermont were represented and distributed as follows: A (11/40, 28%), D (11/40, 28%), B2 (10/40, 25%), B1 (4/40, 10%), E (2/40, 5%), C (1/40, 3%), and F (1/40, 3%).

From our isolates, a representative isolate from each indistinguishable isolate pair was included in further analysis, resulting in 32 fecal isolates included in the comparison. Virulence factors from all virulence factor groups were found in both fecal and invasive isolates, respectively: fimbriae and

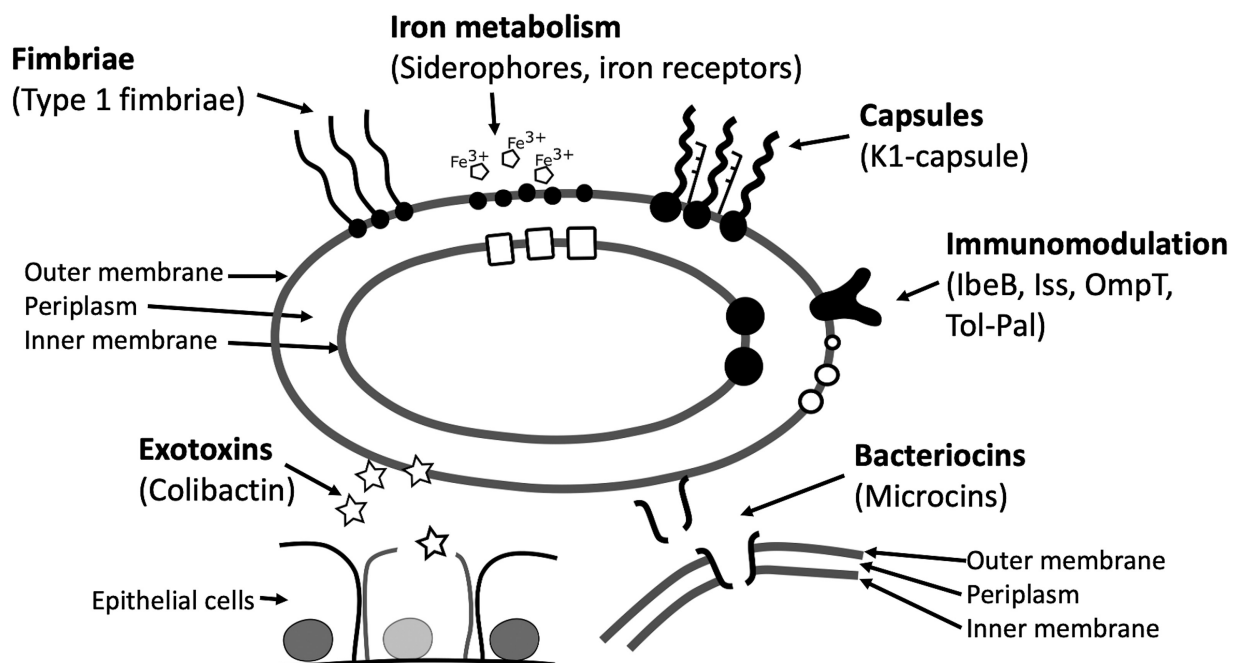
Table 1 Comparison of virulence factor frequency of ESBL-producing *Escherichia coli* isolates from feces with invasive *E. coli* isolates, only statistically significant results were listed

Virulence factor group	Genetic determinants for virulence factors found	ESBL-producing <i>E. coli</i> from feces	Invasive <i>E. coli</i> isolates	OR	p-Value
Fimbriae and adhesins	Type 1 fimbriae	24/32 (75%)	31/32 (97%)	10	<0.05
Iron metabolism	Salmochelin	4/32 (13%)	15/32 (47%)	6	<0.05
	Yersiniabactin	23/32 (72%)	30/32 (94%)	6	<0.05
	Fec	13/32 (41%)	22/32 (69%)	3	<0.05
	Fhu	12/32 (38%)	32/32 (100%)	48	<0.005
	ChuA	6/32 (19%)	27/32 (84%)	22	<0.005
Exotoxins	Colibactin	2/32 (6%)	10/32 (31%)	7	<0.05
Immunomodulation	IbeB	14/32 (44%)	23/32 (72%)	3	<0.05
	Iss	17/32 (53%)	26/32 (81%)	4	<0.05
	OmpT	13/32 (41%)	25/32 (78%)	5	<0.005
	Tol-Pal	22/32 (69%)	32/32 (100%)	14	<0.005
Capsule	K1	4/32 (13%)	12/32 (38%)	4	<0.05
Bacteriocins	Microcin H4	0/32 (0%)	6/32 (19%)	7	<0.05

Abbreviations: ESBL, extended-spectrum β -lactamase; OR, odds ratio.

adhesins 25/67 (37%) versus 29/67 (43%), iron metabolism 12/13 (92%) versus 13/13 (100%), exotoxins 7/19 (37%) versus 9/19 (47%), immunomodulation 15/15 (100%) versus 15/15 (100%), bacteriocins 4/29 (14%) versus 14/29 (48%), and capsule types 2/2 (100%) each. Identical isolates accordingly shared the same virulence factor pattern. No virulence factor was found to be significantly more frequent in fecal isolates overall, with the most notable differences between fecal and invasive isolates being observed in virulence factors targeting iron metabolism and immunomodulation. Significantly less often found in fecal isolates were determinants for the

siderophores salmochelin and yersiniabactin, as well as determinants for the iron acquisition proteins Fec, Fhu, and ChuA. Iron is regarded as essential to *E. coli* causing invasive disease, especially in neonatal meningitis. Likewise, genes encoding proteins involved in translocation of the blood–brain barrier (IbeB), the *iss* determinant and capsule K1 involved in complement resistance, the plasminogen activator OmpT, the cell membrane stabilizing Tol-Pal, and the genotoxin colibactin were statistically significantly less often found in the fecal ESBL-producing isolates ([Table 1](#) and [Fig. 1](#)).

**Fig. 1** Schematic illustration of the different virulence factors for *Escherichia coli*.

Discussion

The fecal screening for ESBL-producing *E. coli* conducted here revealed a relatively high perinatal colonization frequency, with 18% detected in mothers and 5% in newborns within days after delivery. The overall occurrence of virulence factors associated with extraintestinal infections was significantly lower in fecal isolates compared with those from extraintestinal sources. It is reassuring that the ESBL-producing *E. coli* from our study exhibit fewer virulence traits compared with invasive isolates. This is consistent with findings from Rottier et al, where the positive predictive value of fecal carriage of Enterobacteriaceae with ESBL to develop symptomatic infection was relatively low.⁵

The establishment of serious invasive infections in neonates with the opportunistic pathogen *E. coli* is mainly caused by bacterial translocation from the intestine to the bloodstream. The prerequisites for this process are successful bacterial colonization of the gut and effective interaction of the bacteria with the host. Shortly after birth, a newborn rapidly acquires colonization, primarily from the mother's microbiome.⁶ Isolates from phylogroup non-B2 are less frequently associated with invasive infections and are often considered true commensals of the gut. In contrast, *E. coli* from phylogroup B2 not only possesses acquired virulence factors, providing them with the prerequisites for invasiveness but has also been linked to persistent colonization of the intestine and invasive infections.⁷

Screening for ESBL-producing Enterobacteriaceae in neonatal intensive care units is commonly justified to increase awareness for outbreak-preventing strategies, given the catastrophic consequences of uncontrolled spread.⁸ Moreover, adequate treatment of ESBL-producing isolates in an individual can be life-saving when a symptomatic infection arises.⁹ However, the understanding of the risk of symptomatic infection attributable to intestinal carriage of ESBL-producing Enterobacteriaceae remains limited.¹⁰ In recent years, it has been acknowledged that patients and their caregivers may undergo feelings of worry, anxiety, or stigma upon receiving positive test results and guidance from health care providers regarding the implications of ESBL colonization in the gut can facilitate coping.¹¹

Conclusion

All the above underscore the necessity for a deeper understanding of the implications of ESBL-producing Enterobacteriaceae colonization, particularly concerning antimicrobial treatment decisions. Uncertainty in risk assessment of carriage of resistant bacteria may result in unnecessary use of broad-spectrum antibiotics and impact the intestinal microbiome.

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Conflict of Interest

None declared.

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