Emergence of Antibiotic Resistance-Associated Clones Among *Escherichia coli* Recovered From Newborns With Early-Onset Sepsis and Meningitis in the United States, 2008–2009

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Background. *Escherichia coli* associated with early-onset sepsis (EOS) have historically been antibiotic-susceptible and K1-encapsulated. In the era of emerging antibiotic resistance, however, the clonal makeup of *E coli* associated with EOS has not been well characterized.

Methods. *Escherichia coli* isolates were collected from 28 cases of EOS and early-onset meningitis (EOM) from April 2008 through December 2009, during a parent study conducted at National Institute of Child Health and Human Development Neonatal Research Network centers from February 2006 through December 2009. Clinical and microbiologic data were collected for the parent study. We applied polymerase chain reaction- and sequence-based molecular techniques to determine clonal, virulence-associated and antibiotic resistance-associated traits of the *E coli* isolates.

Results. Among 28 *E coli* strains, phylogroup B2 strains predominated (68%), of which more than half were K1-encapsulated (53%). Phylogroup D strains were prominent as well (18%), but none were K1-encapsulated. Across the strain collection, the rate of ampicillin resistance was high (78%). The sole strain resistant to either extended-spectrum cephalosporins or fluoroquinolones represented ST131 *H*30-Rx, the multidrug-resistant subclone that has emerged worldwide in the last decade. This strain encoded extended-spectrum β-lactamase CTX-M-15 and carried an IncF plasmid of type F2:A1:B-.

Conclusions. In this collection of EOS/EOM-associated *E coli* isolates, we observed a high rate of ampicillin resistance, a low rate of fluoroquinolone resistance, and no aminoglycoside resistance, with resistance to third-generation cephalosporins appearing in only a single strain, from the worldwide emerging ST131 clone. Ongoing surveillance of antibiotic resistance among EOS isolates is warranted, to ensure that standard empiric regimens remain effective.

Key words. antibiotic resistance; early-onset sepsis; *Escherichia coli*; ST131.

Extraintestinal infections caused by *Escherichia coli* have historically been associated with a limited number of antibiotic-susceptible clones from phylogenetic groups B2 and D [1]. Newborn meningitis, in particular, has been associated with a limited number of K1 polysaccharide-encapsulated clones from phylogenetic group B2 and D [2]. In the last decade, however, there have been important shifts in the molecular epidemiology of extraintestinal pathogenic *E coli* associated with community-acquired infections, which correspond to increasing rates of resistance to key antibiotics, including fluoroquinolones and extended-spectrum cephalosporins [3]. There are few contemporary studies of the molecular features of *E coli* associated with early-onset neonatal sepsis in the United States [4].

MATERIALS AND METHODS

Isolate Collection

We collected *E coli* isolates from the blood and cerebrospinal fluid (CSF) of infants diagnosed with early-onset sepsis (EOS) and/or early-onset meningitis (EOM) at 16 university based centers of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network. These isolates were collected as a supplement to a larger parent study conducted over the period February 1, 2006 to December 31, 2009 [5]. This supplemental study was added in 2008. Upon approval by the institutional review board at each center, available clinical isolates collected during 2008 and 2009 were shipped from center clinical microbiology laboratories directly to the testing molecular laboratory at University of Washington, Seattle, WA (S.J.W.). Clinical data collected during the parent study included maternal clinical history within 72 hours of delivery and infant gestational age, birth weight, and final outcome (death, discharge). Antibiotic susceptibility data were collected for each isolate when available.

Phylotyping of Host Strains by Multiplex Polymerase Chain Reaction

To assign each *E coli* isolate to 1 of the 4 major phylogenetic groups, we used a rapid, 3-target polymerase chain reaction (PCR) assay in widespread use [6].

Multiple Locus Sequence Typing

We sequenced 7 housekeeping gene loci for all *E coli* isolates [7]. BioNumerics version 6.5 (Applied Maths, Belgium) was used to assemble sequence traces. Allele and sequence typing (ST) assignments were made according to the Achtman scheme (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli, accessed March 16, 2015).

Subclonal Analysis of Same-Sequence Typing Strains

Isolates sharing ST profiles were subjected to sequencing of the *fimH* (type 1 fimbrial adhesin) typing region (nucleotides 64-552; $fimH_{TR}$). Sequencing of $fimH_{TR}$ was carried out, sequences were aligned using BioNumerics (Applied Maths, Belgium), and alleles assigned as previously described [8].

Screening for the Extraintestinal Pathogenic *Escherichia coli* Pathotype

We assessed the capacity for extraintestinal pathogenicity by using a PCR-based protocol screening for presence of confirmed and putative virulence factors. Carriage of 2 or more of 5 virulence markers (*papA* and/or *papC*, *sfa/foc*, *afa-dra*, *iutA*, and *kpsMT*II) has been validated as independently predictive of extraintestinal pathogenic *E coli* (ExPEC) status by statistical analysis of strain collections wherein ExPEC status could be inferred from epidemiologic source or experimental virulence [9]. The housekeeping gene *uidA* serves as a positive control for bacterial template DNA.

Characterization of β-Lactamase Genes

Antimicrobial susceptibility testing was performed as part of routine clinical care at the treating hospitals, as described previously [5]. All clinical isolates were subjected to PCR testing to detect the bla_{TEM} and bla_{CTX-M} enzymes [10]. Sequencing of the positive amplicons was carried out using the same primers used for detection: TEM-A 5 -GAA AGG GCC TCG TGA TAC GC-3 and TEM-B 5 -TCA TCC ATA GTT GCC TGA CTC C-3 [11] and CTX-M-F 5 -CGA TGT GCA GTA CCA GTA A-3 and CTX-M-R 5 -TTA GTG ACC AGA ATC AGC GG-3 [12], respectively. Allele identification was accomplished by alignment to a set of reference sequences (http://www.lahey.org/Studies, accessed March 16, 2015).

Detection of Specific Extraintestinal Pathogenic *Escherichia coli*-Associated Surface Antigens

We applied a subset of primers from the complete set described for detection of *rfb* fragments characteristic of 12 common ExPEC-associated O antigens [13]; specifically, we used primers to detect O1, O2, and O18 antigens that are characteristic of ST95 strains [7,14]. Then, carriage of a K1 capsular biosynthesis determinant was detected by PCR using primer pair *kpsMT*-K1-F 5 -TAG CAA ACG TTC TAT TGG TGC-3 and *kpsMT*-II-R 5 -CAT CCA GAC GAT AAG CAT GAG CA-3 , as described by Johnson and Stell [15].

Limited Polymerase Chain Reaction-Based Replicon Typing and Replicon Sequence Typing (RST)

The plasmid content of wild-type strains was evaluated by a subset of PCR-based replicon typing primers that target essential plasmid replication genes; specifically, we used primers designed to identify the IncFIIA, IncFIA, and IncFIB replicons associated with IncF plasmid backbones [16]. For the single bla_{CTX-M}-positive strain, we used the IncFIIA and IncFIA detection primers to perform replicon sequence typing, as previously described [17].

RESULTS

Study Population

Isolates from 28 of the 107 neonates diagnosed with *E coli* EOS in the parent study were characterized in depth. Maternal and infant characteristics of the infants whose isolates were studied were similar to those of the infants not included in the isolate study (Supplemental Table 1).

Clinical and Microbiologic Features

We recovered 32 *E coli* isolates (30 from blood, 2 from CSF) from 28 patients. Four patients provided 2 isolates

each, including 2 patients that provided 2 isolates from blood (1 patient on different days, the other on the same day) and 2 patients that provided 1 isolate each from blood and CSF. Thus, meningitis represented 2 of 28 cases (7%) from which clinical isolates were analyzed; similarly, meningitis represented 5 of 79 cases (6%) of early-onset *E coli* disease among the cases not analyzed (Supplemental Table 1). The majority of cases analyzed and cases not analyzed had mothers who received antibiotics within 72 hours before delivery (78% in each group), with ampicillin given to over 70% of those who received antibiotics. In both groups, rates of resistance to ampicillin were high (78% of isolated strains, 79% of parent study cases not included in the isolate study). A majority of ampicillin-resistant isolates occurred in infants whose mothers received ampicillin within 72 hours of delivery (11 of 21 ampicillin-resistant strains studied; 40 of 59 not studied). However, in the overall group of 102 *E coli* isolates tested [5], ampicillin resistance was not more likely among infants whose mothers received intrapartum ampicillin than among those who did not (85% vs 69%, $P = .085$). Resistance to third-generation cephalosporins was limited (4% of isolated strains, 3% of parent study cases not included in the isolate study). Gentamicin resistance was not detected among the collected isolates but occurred in 5% of the parent study cases not analyzed. Overall, the features of the cases with analyzed isolates were similar to those of parent study cases.

Clonal Properties of Paired Isolates

All 32 *E coli* isolates were subjected to rapid phylotyping and multiple locus sequence typing (MLST). For the 4 patients yielding 2 isolates, phylogenetic and MLST profiles were identical within each pair, strongly suggesting that each patient was infected with a single strain only. Subsequent results are thus described for 28 strains, 1 from each patient; the chronologically earlier isolate was selected for testing from pairs.

Phylogroup Distribution and Multiple Locus Sequence Typing Analysis

The isolate collection was dominated by strains from phylogroup B2 (19 of 28, 68%; Table 1), with a lesser contribution of strains from phylogroup D (5 of 28, 18%). All 20 premature infants had strains from phylogroup B2 or D compared with 4 of 8 (50%) of the term infants ($P = .003$). The remaining strains represented phylogroups A and B1 (4 of 28, 14%); all 4 were collected from term infants. Fourteen distinct ST profiles were observed (Table 1), including 9 from phylogroup B2, 1 from phylogroup D, and 4 from phylogroups A and B1. Six STs contained 2 or more representatives, including ST95-B2 (6 isolates), ST69-D (5 isolates), ST420-B2 (3 isolates), ST12-B2, ST80-B2,

and ST131-B2 (2 isolates each); each of the remaining 8 ST profiles was represented by a single strain. Early-onset meningitis case strains derived from ST95-B2 and ST12-B2.

Virulence Properties

The ExPEC pathotype indicating virulence in extraintestinal compartments (urine, blood, CSF) was detected in 18 of 28 (64%) strains, including 13 of 19 (63%) phylogroup B2 strains (including both EOM strains), 5 of 5 phylogroup D strains, and 0 of 4 phylogroup A and B1 strains. Specifically, ExPEC status was detected in all 6 ST95-B2 strains, all 5 ST69-D strains, 1 of 2 ST131-B2 strains, both ST12-B2 strains, and both ST80-B2 strains, but 0 of 3 ST420-B2 strains. A greater proportion of the isolates from premature infants, all of whom had strains from phylogroups B2 or D, had a virulence factor score of 2 or more compared with those from term infants, respectively 15 of 20 (75%) vs 3 of 8 (38%; $P = .09$). The specific factors varied in prevalence: *kii*, 22 of 28 (79%); *papA* or *papC*, 16 of 28 (57%); *iutA*, 13 of 28 (46%); and *sfa/foc*, 5 of 28 (18%).

K1 encapsulation, which is implicated in the pathogenesis of EOS and meningitis [18], was detected in 11 of 28 (39%) strains, including 10 of 19 (53%) phylogroup B2 strains, in 0 of 5 phylogroup D strains, and 1 of 4 (25%) phylogroup A and B1 strains. Specifically, K1 encapsulation was detected in 4 of 6 ST95-B2 strains and 3 of 3 ST420-B2 strains, but none of 5 ST69-D strains, 2 ST12-B2 strains, 2 ST80-B2 strains, or 2 ST131-B2 strains. Only 1 of 2 EOM strains (ST95-B2, but not ST12-B2) was K1 encapsulated.

Antibiotic Resistance-Associated Properties

Of 27 strains with susceptibility testing results available from the parent study dataset, ampicillin resistance was present in 21 (78%) strains, whereas gentamicin resistance was not detected at all. On molecular testing, the bla_{TEM} β-lactamase was detected in 22 of 28 (79%) strains, including 20 of 21 (95%) ampicillin-resistant strains, 1 of 6 (17%) ampicillin-susceptible strains, and 1 strain not tested for ampicillin susceptibility; all TEM amplicons were confirmed by sequencing to represent narrow-spectrum TEM-1 ampicillinases (data not shown). Resistance to extended-spectrum (ie, third generation) cephalosporins was present in 1 of 26 strains tested (strain NRN04, from ST131-B2); this also represented the only strain in which the extended-spectrum β-lactamase (ESBL)-encoding bla_{CTX-M} gene was detected. Extended-spectrum β-lactamase-positive NRN04 was 1 of the 21 strains resistant to ampicillin and was also the only strain in the collection resistant to both ciprofloxacin and trimethoprim-sulfamethoxazole. The NRN04 isolate was identified in blood taken from a preterm infant within 1 hour after birth. The mother had received ampicillin and gentamicin for fever, chorioamnionitis (confirmed

Table 1. Phylogenetic, Clonal, Molecular, and Antibiotic Resistance-Associated Properties of 28 *E coli* Recovered From Early-Onset Sepsis, 2008–2009[a]

Strain	ST	fimH	O-Ag	K1	ExPEC	VF Profile	AMP	ESC	CIP	T/S	TEM-1	CTX-M	IncF Replicons
Phylogroup B2 (19 isolates)													
NRN28[b]	ST95	18	O18	+	+	papAC, sfa, kii	R	S	S	R	+	-	FII
NRN06	ST95	27	O2	-	+	papAC, iutA, kii	R	S	S	S	+	-	FII, FIB
NRN24	ST95	30	O1	-	+	papAC, iutA, kii	R	S	S	S	+	-	FII, FIB
NRN11	ST95	41	O1	+	+	papAC, iutA, kii	S	S	S	n.t.	-	-	FII, FIB
NRN22	ST95	41	O1	+	+	papAC, iutA, kii	R	S	S	n.t.	+	-	FII, FIA, FIB
NRN29	ST95	41	O2	+	+	papAC, kii	S	S	S	S	-	-	FII, FIB
NRN13	ST420	5	n.t.	+	-	kii	R	S	S	S	+	-	FII, FIB
NRN27	ST420	5	n.t.	+	-	kii	R	S	S	S	+	-	FII, FIB
NRN23	ST420	5	n.t.	+	-	kii	S	S	n.t.	S	-	-	FII, FIB
NRN14	ST12	5	n.t.	-	+	papAC, sfa	R	S	S	S	+	-	FII
NRN21[b]	ST12	5	n.t.	-	+	papAC, iutA	R	S	S	n.t.	+	-	FII, FIB
NRN08	ST80	1	n.t.	-	+	sfa, kii	R	S	S	S	+	-	-
NRN09	ST80	1	n.t.	-	+	sfa, kii	R	S	S	S	+	-	-
NRN04	ST131	30	n.t.	-	-	kii	R	R	R	R	+	+	FII, FIA
NRN03[c]	ST131	22	n.t.	-	+	papAC, iutA, kii	R	S	n.t.	S	+	-	FII, FIB
NRN05	ST73	n.t.	n.t.	-	+	papAC, sfa, kii	S	n.t.	S	S	+	-	-
NRN02	ST144	n.t.	n.t.	+	+	papAC, iutA, kii	R	S	S	S	+	-	FII, FIA, FIB
NRN20	ST372	n.t.	n.t.	+	-	kii	R	S	S	n.t.	+	-	-
NRN17	ST538	n.t.	n.t.	+	-	kii	R	S	S	S	+	-	FII, FIB
Phylogroup D (5 isolates)													
NRN16	ST69	27	n.t.	-	+	papAC, iutA, kii	n.t.	n.t.	n.t.	n.t.	+	-	FII, FIB
NRN18	ST69	27	n.t.	-	+	iutA, kii	R	S	S	R	-	-	FII, FIA, FIB
NRN01	ST69	27	n.t.	-	+	papC, iutA, kii	R	S	S	R	+	-	FII, FIB
NRN19	ST69	27	n.t.	-	+	papAC, iutA, kii	R	S	n.t.	S	+	-	FII, FIB
NRN26	ST69	27	n.t.	-	+	papAC, iutA	R	S	S	S	+	-	FII, FIB
Phylogroup A or B1 (4 isolates)													
NRN10	ST540	n.t.	n.t.	-	-	papC	R	S	n.t.	S	+	-	FII
NRN07	ST607	n.t.	n.t.	-	-	-	S	S	S	S	-	-	-
NRN12	ST1507	n.t.	n.t.	+	-	kii	S	S	S	S	-	-	-
NRN15[c]	ST2222	n.t.	n.t.	-	-	iutA	R	S	S	R	+	-	FII, FIB

Abbreviations: AMP, ampicillin; CIP, ciprofloxacin; CSF, cerebrospinal fluid; CTX-M, extended-spectrum beta-lactamase; ESC, extended-spectrum cephalosporin (cefotaxime, ceftriaxone, or ceftazidime); ExPEC, extraintestinal pathogenic *E coli* pathotype (- for 0-1 virulence factors, + for 2 or more virulence factors); *fimH*, type 1 fimbrial adhesin *fimH* typing region allele; K1, K1 polysaccharide capsule; n.t., not tested; O-Ag, somatic antigen; R, resistant; S, susceptible; ST, sequence type; TEM-1, narrow-spectrum ampicillinase; T/S, trimethoprim/sulfamethoxazole; VF profile, virulence factors detected.
[a]No isolate was resistant to gentamicin.
[b]Second isolate from CSF.
[c]Second isolate from blood: +, detected; -, not detected.

by histology), and premature rupture of membranes, within 2 hours before delivery.

Plasmid-Associated Properties

Because of the well documented relationship between ExPEC clones and IncF-related plasmids [16, 19, 20], we screened the collected strains for carriage of plasmids from the IncF family. Twenty-two of 28 (79%) strains demonstrated carriage of an IncF plasmid, including 15 of 19 (79%) phylogroup B2 strains, 5 of 5 phylogroup D strains, and 2 of 4 phylogroup A and B1 strains; as well as 18 of 22 (82%) TEM-1-positive strains and 4 of 6 (67%) TEM-1-negative strains. IncF-related profiles included FII only (3 strains), along with hybrid profiles FII-FIB (15 strains), FII-FIA-FIB (3 strains), and FII-FIA (1 strain).

Additional Molecular Features of Same-Sequence Typing Strains

Because antigenic and virulence-associated properties can vary within ST lineages [8, 14, 21], we performed $fimH_{TR}$ sequencing on same-ST strains for additional phylogenetic resolution. The 5 strains of ST69-D were indistinguishable from one another, as were 3 strains from ST420-B2, and 2 strains each from ST12-B2 and ST80-B2 (Table 1). However, within both ST95-B2 and ST131-B2, $fimH_{TR}$ variability was seen: 4 alleles were noted among 6 ST95 strains, and alleles differed between 2 ST131 strains.

Because the 4 $fimH_{TR}$ alleles detected in ST95 matched those seen in a previous collection of serotyped ExPEC isolates [8], we performed PCR-based screening for somatic (lipopolysaccharide, O) antigens prominent among ExPEC collections. The observed results confirmed previously reported relationships between $fimH_{TR}$ and O antigens within ST95: $fimH_{TR}18$ with O18, $fimH_{TR}27$ with O2, $fimH_{TR}30$ with O1, and $fimH_{TR}41$ with both O1 and O2 [8].

For ST131 strain NRN04, we confirmed by sequencing that the bla_{CTX-M} amplicon represented ESBL variant CTX-M-15, and the strain background corresponded to a specific $fimH_{TR}$ lineage (*H30*), as previously reported

[22, 23]. In addition, sequencing of IncF-related FII and FIA amplicons from NRN04 revealed that this ST131 strain carried an F2:A1:B- plasmid type, similar to that carried by model strain EK499, the so-called "epidemic strain A" [24].

Clinical Outcomes

All 4 term infants with low-virulence, phylogroup A or B1 *E coli* strains and 3 of 4 term infants with high-virulence, phylogroup B2 or D strains survived to discharge (Supplemental Table 2). Most (70%) of the 20 premature infants with high-virulence strains survived also, including the infant infected by ESBL-producing, multidrug-resistant ST131-B2 strain NRN04; this child was treated with meropenem and gentamicin. Five infants (4 preterm, 1 term) were infected by strains of antibiotic-resistant clone ST69-D [25], which demonstrated resistance to ampicillin (4 of 4 tested) and trimethoprim-sulfamethoxazole (2 of 4 tested); 3 of these infants, all preterm, died.

DISCUSSION

As part of a parent study of EOS conducted at NICHD Neonatal Research Network hospitals [5], we collected and analyzed clinical *E coli* isolates from 28 cases of EOS and EOM treated in 2008 and 2009. This study produced 3 main observations. First, recent EOS isolates of *E coli* were (1) similar to earlier EOS collections with regard to prevalence of K1 encapsulation and (2) similar to other ExPEC with regard to phylogroup B2 predominance. Second, 78% of study isolates were resistant to ampicillin, but none were resistant to gentamicin. Finally, prominent clones associated with the emergence of multidrug resistance across the globe (including highly virulent sequence type 131, which has driven the spread of extended-spectrum β-lactam resistance) were detected in several patients.

The high rate of ampicillin resistance (78%) observed in this EOS strain collection from 2008 to 2009 is consistent with the rate reported among EOS *E coli* isolates (76%) collected from 2005 to 2008 by the Active Bacterial Core surveillance program [26]; both values are well above the rate of ampicillin resistance (51.6%) recently reported in a collection of 1679 adult extraintestinal *E coli* isolates [27]. The frequency of ampicillin resistance in our study isolates was matched by rates of bla_{TEM-1} ampicillinase and IncF plasmid detection (both 79%), suggesting that IncF plasmid-borne bla_{TEM} genes are responsible for the clinical phenotypes observed. Indeed, carriage of bla_{TEM-1} on plasmids of the IncF family has been appreciated for many years [28]. IncF plasmids often also encode several extraintestinal virulence factors (including

siderophores and serum survival determinants) and are prominent among ExPEC [17], but it is unclear whether the relative enrichment of ampicillin resistance in this collection is driven by acquisition of bla_{TEM} by clonally stable IncF plasmids, wholesale replacement of bla_{TEM}-negative IncF plasmids by emerging bla_{TEM}-positive IncF plasmids, or other dynamics.

More worrisome epidemiologic changes were reflected by the detection of 2 clones associated with the worldwide emergence of multidrug-resistance in *E coli*: ST69-D and ST131-B2. ST69 was first described in 2000 as a cause of antibiotic-resistant, community-acquired urinary tract infections in adults [29]. More recently, ST131 (O25:H4, phylogroup B2) has been associated with the explosive increase of multidrug—and extended-spectrum cephalosporin—resistance in community-associated extraintestinal infections worldwide [30], appearing domestically in pediatric populations as early as 2003 and increasing steadily in frequency thereafter [23]. A specific subclone of ST131, defined by carriage of the *fimH*30 allele and marked by chromosomally determined fluoroquinolone resistance, has been identified as a major contributor to the epidemiologic shift towards multidrug-resistance in Gram-negative pathogens worldwide [31, 32]. As far as we are aware, this is the earliest CTX-M-15-positive *E coli* ST131 reported in newborn bacteremia in the United States. The appearance of multidrug-resistant clones in neonatal *E coli* isolate collections is a worrisome indicator of the ecologic and pathogenic versatility of these menacing bacteria.

Collections of *E coli* associated with extraintestinal (urinary tract and bloodstream) infections are typically dominated by strains from phylogroup B2 [1]. In a large study of community-acquired, urinary-source *E coli* bacteremia across France, for example, phylogroup B2 strains accounted for 64% and 63% of isolates from children and adults, respectively [33]. Likewise, the assorted virulence factors associated with extraintestinal disease (polysaccharide capsules, toxins, siderophores, fimbriae) are more frequently found in strains from phylogroup B2 (and to a lesser extent, phylogroup D) than in those from phylogroups A and B1 [34]. In the present EOS/EOM collection, however, specific virulence factors were less prevalent (*papA* or *papC*, 57%; *iutA*, 46%; *sfa/foc*, 18%) than they were in isolates of children with community-acquired, urinary source bacteremia (86%, 79%, and 28%, respectively [33]), reflecting the relative fragility of the present study population. Thus, the phylogroup distribution and virulence factor composition of this strain collection reflect the variable balance between bacterial and host factors in distinct patient populations.

After observations of a limited number of *E coli* strain types (such as serotypes O18:K1:H7 and O7:K1 [2]) from newborn disease worldwide, the specific association between K1 encapsulation and newborn meningitis was described by McCracken et al [35] and Robbins et al [36] almost 40 years ago. Although K1 encapsulation rates up to 81% have been reported among newborn meningitis isolates [37], lower rates are typically seen in newborn sepsis collections, where meningitis remains a relatively infrequent presentation. For example, among 24 newborn sepsis isolates recovered between 2006 and 2013 at a single US center by Shakir et al [4], 50% of isolates were K1-encapsulated. In the present collection, K1 capsular determinants were detected in 39% of isolates overall, closer to the rate of 34% observed among US adult cystitis isolates [38]. The observation that 1 of the 2 meningitis-associated strains in this collection was K1 encapsulated and represented canonical newborn meningitis-associated serotype O18:K1:H7 [2, 14, 39] indicates that traditional clones of *E coli* have continued to circulate into the 21st century, with virulence intact.

This study has a number of limitations. Repeat antibiotic susceptibility testing was not performed with the collected isolates to confirm the findings of the primary clinical microbiology laboratories, allowing for under- or overdetection of specific resistance traits. Next, molecular analysis to detect relevant antibiotic resistance genotypes was limited only to bla_{TEM} and bla_{CTX-M} genes. Furthermore, causation of the relevant phenotypes was not confirmed by plasmid isolation from the wild-type isolates, allowing for possible misattribution of phenotypic features to the assayed genes or plasmid types. Finally, with regard to the parent study, we have tested isolates from only a limited subset of cases, and although the resistance phenotypes of the tested subset are similar to those of the parent cohort, we cannot confirm that the genotypic findings in the subset also apply to the parent cohort. However, in light of consistency between our observations and previously reported molecular epidemiologic studies, we believe that the stated shortcomings do not substantially detract from the findings and conclusions of our study.

CONCLUSIONS

Thus, although rates of ampicillin resistance remained high and multidrug-resistant clones appeared among EOS/EOM isolates of *E coli*, empiric antibiotic combinations appear to have remained appropriate in the United States even through the initial worldwide surge in multidrug resistance. Continued vigilance is warranted for individual patients and populations alike.

Supplementary Data

Supplementary materials are available at the *Journal of the Pediatric Infectious Diseases Society* online (http://jpids. oxfordjournals.org).

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