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ORIGINAL ARTICLE

Escherichia coli early-onset sepsis: trends over two decades

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Abstract *Escherichia coli* early-onset sepsis (EOS) is an important cause of mortality and morbidity in neonates, especially in preterm and very low birth weight (VLBW) newborns. The aim of our study was to evaluate potential changes in the clinical and microbiological characteristics of *E. coli* EOS in our setting. Epidemiological, clinical, and microbiological data from all neonates with proven *E. coli* EOS from January 1994 to December 2014 were retrospectively collected in a single tertiary care hospital in Barcelona (Spain). Seventy-eight *E. coli* EOS cases were analyzed. A slight increase in the incidence of *E. coli* EOS was observed during the study period. VLBW newborns remained the group with higher incidence (10.4 cases per 1000 live births) and mortality (35.3%). Systematic use of PCR increased *E. coli* EOS diagnosis, mainly in the term newborn group. There was an increase in resistant *E. coli* strains causing EOS, with especially high resistance to ampicillin and gentamicin (92.8 and 28.6%, respectively). Nonetheless, resistant strains were not associated with poorer clinical outcomes.

Conclusions: There is an urgent need to reconsider the empirical therapy used in neonatal EOS, particularly in VLBW newborns.

What is Known:
- *E. coli* early-onset sepsis (EOS) and *E. coli* resistant strains have been described as overall stable but increasing in VLBW neonates (< 1.500 g) in previous studies.

What is New:
- Our study shows an increasing incidence of *E. coli* EOS in all age groups, overruling group B *Streptoccocus* for the last 10 years. *E. coli* resistant strains also increased equally in all age groups, with high resistance rates to our first line antibiotics (ampicillin and gentamicin).
- Empiric antibiotic therapy of EOS, mainly in VLBW newborns, should be adapted to this new scenario.

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Abbreviations

CNS	Central nervous system
CSF	Cerebroespinal fluid
E. coli	*Escherichia coli*
EOS	Early-onset sepsis
ESBL	Extended-spectrum beta-lactamase
GBS	Group B *Streptoccocus*
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
VLBW	Very low birth weight

Introduction

Early-onset sepsis (EOS) is a major cause of mortality and morbidity in neonates, particularly preterm newborns and those with a very low birth weight (VLBW) [29]. After the publication of the Centers for Diseases Control and Prevention (CDC) recommendations for intrapartum antibiotic prophylaxis against group B *Streptococcus* (GBS) in 1996, the global incidence of GBS EOS dramatically decreased, although it still remains the first EOS cause [24]. *Escherichia coli,* the most frequent EOS cause after GBS, became a prominent pathogen in this setting, causing higher morbidity and mortality rates than those of GBS [20]. Preterm delivery, low birth weight, intrapartum fever, chorioamnionitis, and preterm and prolonged membrane rupture are considered risk factors for *E. coli* EOS [29].

The overall incidence of *E. coli* EOS seems to have remained stable in the last few decades [1, 3, 4, 20, 27]. To our knowledge, only 2 studies have used stratified samples, and these found an increased incidence limited to VLBW infants [4, 27]. Stoll et al. demonstrated a rise in the number of *E. coli*-related EOS cases in this subgroup of neonates between 1991 and 1993, and 1998 and 2000 (from 3.2 to 6.8 per 1000 VLBW live births, $p = 0.004$) [27]. Bizzarro et al. showed a similar increase from 1979 to 2006 (from 2.83 to 7.21 per 1000 VLBW live births, $p < 0.001$) [4]. However, in both studies, the number of patients included was relatively low (61 and 53, respectively).

Microbial culture from blood or other sterile fluids is the gold standard in the diagnosis of neonatal sepsis. However, its sensitivity is low due to several reasons: low bacterial load in neonatal blood, small inoculation volume in culture bottles, and the use of intrapartum antibiotic [6, 23]. Thereafter, different molecular techniques have been tried to improve the diagnosis of neonatal sepsis. Amplification methods (conventional and real-time PCR assays) have been tested in several neonatal studies with satisfactory results [8, 14, 16].

There is substantial concern about whether the increasingly more extensive use of intrapartum antibiotics may affect the incidence of antimicrobial-resistant infection. An estimated 65% of VLBW neonates are currently exposed to antibiotics before delivery [26]. Most studies have shown a rise in EOS cases caused by *E. coli* with antimicrobial resistance [10], particularly ampicillin resistance, and in the preterm newborn population [1, 4, 13, 27]. In addition, some studies have found a straightforward relationship between maternal antibiotic use and ampicillin-resistant *E. coli* EOS in the VLBW group [4, 29].

This study was conducted to evaluate potential changes in the clinical and microbiological characteristics of *E. coli* EOS in our setting over the past two decades with the purpose of determining whether our current management protocols are adequate for this population.

Patients and methods

Study design

Hospital Universitari Vall d'Hebron in Barcelona (Spain) is a tertiary care hospital that performs a yearly average of 3800 deliveries and is equipped with a 70-bed newborn intensive care unit (NICU) for infants with complex medical and surgical conditions.

Cases were identified from our local microbiology database. All cases of *E. coli* detection in blood and/or CSF from neonates were analyzed, and included if they fulfilled the proven EOS definition. The epidemiological, clinical, and microbiological data from all consecutive cases of proven *E. coli* EOS from January 1994 to December 2014 were retrospectively collected from electronic and paper medical records depending on the period. Gestational, delivering and postpartum data of the mothers of all the included newborn were also analyzed.

The 21-year period was divided into 4 time intervals for the study (1994–1999, 2000–2004, 2005–2009, and 2010–2014). Newborns were stratified according to gestational age and birth weight.

Data about GBS EOS and annual deliveries stratified by gestational age and birth weight were only available from 2005 to 2014.

Definitions

Proven EOS was established based on *E. coli* detection by culture or polymerase chain reaction (PCR) assay in a normally sterile sample (blood or cerebrospinal fluid [CSF]) obtained from a neonate within 3 days of life or from a neonate within 3

to 7 days of life whose mother's cultures were also positive [17]. Thereafter, patients were classified according to their clinical status as having the following:

E. coli bacteremia: *E. coli* isolated by culture or detected by PCR in a blood sample obtained from an asymptomatic newborn.
E. coli sepsis: *E. coli* isolated by culture or detected by PCR in a normally sterile sample obtained from a newborn with systemic infection-related symptoms.
E. coli septic shock: *E. coli* isolated by culture or detected by PCR in a normally sterile sample obtained from a newborn requiring mechanical ventilation or inotropic support in the setting of clinical sepsis without other recognizable cause and/or coagulopathy.
E. coli meningitis: *E. coli* detected by culture or PCR in CSF [15].

Associated risk factors (preterm delivery, prolonged rupture of membranes, maternal intrapartum fever, and chorioamnionitis) were defined as reported elsewhere [17].

Intrapartum antibiotics were defined as antibiotics started after rupture of membranes and/or uterine contractions. The standard of care was ampicillin plus gentamicin for prolonged rupture of membranes, penicillin for GBS colonization and erythromycin for preterm rupture of membranes. There were no changes in antibiotic management during the 21-year period.

Single-dose cefazolin previous to cesarian section was not considered as intrapartum antibiotic for the purpose of our study, due to the short interval between administration and delivery.

Microbiology

E. coli was isolated from neonatal blood and CSF, and from maternal samples (placenta, vaginal exudate, blood and amniotic fluid) using standard laboratory methods. Antibiotic sensitivity testing was carried out for ampicillin, cefotaxime, imipenem, gentamicin, and amikacin according to the Clinical and Laboratory Standard Institute (CSLI) guidelines from 1994 to 2013 and according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines in 2014. Antibiotic sensitivity testing was performed on strains isolated from all blood culture-positive samples (64). One antibiogram from a strain obtained by culturing a placental sample from a mother whose newborn was diagnosed by PCR was also included.

Starting in 2008, real-time PCR (Progenie, Valencia, Spain) using the SmartCycler system (Cepheid, Sunnyvale, California, USA) was routinely performed to detect *E. coli,* GBS and *Listeria monocygotenes* from neonatal blood and CSF of all neonates with suspicion of early onset sepsis.

Statistical analysis

Since molecular techniques were only available from 2008, incidences were calculated without those newborns that were diagnosed by PCR to avoid selection bias. Moreover, outborn newborns were also excluded for this purpose.

The STATA v13.1 statistical software package was used for the data analysis. Categorical variables are expressed as frequency and percentages. Relationships between the periods and other qualitative variables were analyzed with the chi-square test. Incidence rates were calculated as the number of cases per 1000 live births per period. Poisson's regression was used to compare the incidence between periods. Quantitative variables are expressed as the mean and range.

Ethical aspects

The Ethics Committee of HUVH approved the study in May, 2015 (PR(AMI)158/2015).

Results

Study population and rates

During the period of the study, 79,813 live neonates were delivered at our hospital. Seventy-eight episodes of *E. coli* EOS in 78 newborns were analyzed. Sixty-three of them (80.7%) were born in our center. The other 15 (19.2%) were born in other hospitals and referred to our center due to maternal or neonatal pathology.

Twenty-four (30.8%) cases occurred in term neonates and 54 (69.2%) in preterm neonates. Among the latter, there were 34 (43.6%) VLBW newborns.

The overall incidence of *E. coli* EOS diagnosed by positive blood culture was 0.66 cases per 1000 live births. There was a slight increase in the incidence over time, particularly from the second period to the last one (from 0.29 per 1000 live births during 2000–2004 to 0.82 during 2010–2014) (Table 1). The increase was due to a rise in the term and VLBW group, but did not reach statistically significance in neither of them (Table 2).

Nevertheless, since 2008, when systematic use of PCR was implemented, there was an increase in *E. coli EOS* diagnosis, especially in term newborn (Table 2). This rise was mainly due to an increase of the diagnosis in the term newborn group, with 8 of them being diagnosed only by positive PCR in blood or CSF with negative blood and/or CSF culture.

In the last 10 years, the overall incidence of GBS EOS was 0.50 cases per 1000 live births due to a reduction among preterm newborn cases (from 3.28 per 1000 VLBW live births in 2005–2009 to 1.23 thereafter; and from 1.04 per 1000

Table 1 Incidence of *E. coli* EOS diagnosed by blood and/or CSF culture by time periods

Period	*E. coli* EOS	Live births	Rate per 1000 live births
1994–1999	21	20,997	*1.00*
2000–2004	6	20,984	*0.29*
2005–2009	13 (+1)	21,913	*0.59*
2010–2014	13 (+9)	15,919	*0.82*
Overall (1994–2014)	*53 (+10)*	*79,813*	*0.66*

E. coli EOS Escherichia coli early-onset sepsis, *( )* cases diagnosed only by positive PCR in blood and/or CSF are included in parentheses and not used to calculate incidence rates. Outborn cases are not included

preterm > 1500 g live births to 0.00). Of note, when comparing the incidence of *E. coli* and GBS EOS over the last 10 years, *E. coli* emerged as the first overall cause of EOS (0.69 vs 0.50 per 1000 live births). However, GBS remained the first cause of EOS in the term group (0.39 vs 0.06 per 1000 live births).

Noteworthy, during the last period (2010–2014), the systematic use of PCR caused an increase in the diagnosis of E. coli EOS so the same number of *E.coli* and GBS EOS was observed in term newborns (10 cases for each pathogen) (Table 2).

Maternal data

Seventy-eight mothers of 78 cases of *E. coli* EOS were analyzed, from 1 week before delivery and up to 3 days postpartum. Seventeen mothers (21.5%) had *E. coli* infection during pregnancy, diagnosed by isolation of *E. coli* in vaginal

Table 2 Incidence of *E. coli* and GBS EOS diagnosed by blood and/or CSF culture, stratified by newborn gestational age and birthweight, between 2005 and 2014

	2005–2009	2010–2014	Overall (2005–2014)
All age groups			
Live births	21,913	15,919	37,832
E. coli EOS	13 (+1)	13 (+8)	26 (+9)
E. coli EOS rate per 1000 live births	0.59	0.82	0.69
GBS EOS	12 (+1)	7 (+4)	19 (+5)
GBS EOS rate per 1000 live births	0.55	0.44	0.50
VLBW preterm newborns			
Live births	916	810	1726
E. coli EOS	9	9	18
E. coli EOS rate per 1000 VLBW live births	9.83	11.11	10.43
GBS EOS	3 (+1)	1	4 (+1)
GBS EOS rate per 1000 VLBW live births	3.28	1.23	2.32
Preterm newborns > 1500 g			
Live births	2872	2174	5046
E. coli EOS	4	2	6
E. coli EOS rate per 1000 preterm > 1500 g live births	1.39	0.92	1.19
GBS EOS	3	0	3
GBS EOS rate per 1000 preterm > 1500 g live births	1.04	0	0.59
Term newborns			
Live births	18,125	12,935	31,060
E. coli EOS	0 (+1)	2 (+8)	2 (+9)
E. coli EOS rate per 1000 live births	0.00	0.16	0.06
GBS EOS	6	6 (+4)	12 (+4)
GBS EOS rate per 1000 live births	0.33	0.46	0.39

E. coli EOS Escherichia coli early-onset sepsis, *GBS EOS* group B *Streptococcus* early-onset sepsis, *VLBW* very low birthweight, *NA* data not available, ( ) cases diagnosed only by positive PCR in blood and/or CSF are included in parentheses and not used to calculate incidence rates. Outborn cases are not included

Table 3 Maternal data

Maternal data (n = 78)	Result	Percent
Age	30 years (17–42)	
GBS screening (positive/performed)	8/44	10.26/56.41
Cesarean section delivery	28	35.90
E. coli infection during gestation	17	21.79
Risk factors for EOS	70	89.74
- Preterm delivery	54	69.23
- Prolonged rupture of membranes	42	53.85
- Suspected chorioamnionitis	30	38.46
- Maternal intrapartum fever	25	32.05
Intrapartum antibiotic	50	64.10
Maternal cultures (positive/performed)		
- Placenta	30/34	38.46/43.60
- Vaginal exudate	19/21	24.36/26.92
- Blood	6/16	7.69/20.51
- Amniotic fluid	1/5	1.82/6.41

E. coli Escherichia coli, *GBS* group B *Streptococcus*, *EOS* early-onset sepsis

exudate or urinary samples plus compatible clinical symptoms. All had been treated with antibiotics.

Almost all the mothers (88.6%) had risk factors for perinatal infection. Fifty-seven (72.2%) had received intrapartum antibiotics for a history of a gestational infection, maternal risk factors for neonatal sepsis, or GBS colonization. The mothers' samples (blood, placenta, vaginal exudates and amniotic fluid) were cultured in 36 cases (45.5%), due to premature rupture of membranes or clinical suspicion of maternal or neonatal infection; and 34 were positive for *E. coli.* Data about placental histological analysis were not available for this study.

Complete data on the maternal characteristics and delivery are shown in Table 3.

Neonatal data

Seventy-eight cases of *E. coli* EOS were analyzed. Mean gestational age was 32 weeks (range 23–42 weeks). Median gestational age was 31 weeks (IQR 27–36 weeks). Fifty-four cases (69.6%) occurred in preterm newborns: 22 (28.2%) had been born at less than 28 weeks, 19 (24.4%) between 28 and 32 weeks, and 13 (16.7%) between 33 and 37 weeks. Mean weight was 2006 g (range, 430–4340 g). Thirty-four were VLBW newborns, accounting for 43.5% of all cases and 62.5% of preterm infants. Seventeen VLBW babies (48.6%) weighed less than 1000 g. The clinical and microbiological features of the 78 neonates with *E. coli* EOS are described in Table 4.

Blood cultures were positive in 64 cases (82.1%). The other 14 cases were diagnosed by positive PCR findings in blood and/or CSF samples. Blood and/or CSF were also cultured and were negative. *E. coli* PCR had been performed in 33 newborns (41.7%): 27 in blood and 25 in CSF samples, with positive findings in 13 and 8 samples, respectively.

Meningitis was diagnosed in 10 neonates, 9 of them were term newborns. Lumbar puncture was not carried out in 34 newborns (43.6%), mainly preterm; who were clinically unstable.

Table 4 Newborns' clinical and microbiological data

	Total (n = 78)	Term newborns (n = 24)	Preterm newborns	
			> 1500 g (n = 20)	< 1500 g (n = 34)
Clinical features				
- Septic shock	21 (26.92)	3 (12.50)	6 (30.00)	12 (35.29)
- Sepsis	41 (52.56)	13 (54.17)	10 (50.00)	18 (52.94)
- Bacteremia	15 (19.23)	10 (41.67)	4 (20.00)	1 (2.94)
- Unknown	1 (1.28)	0 (0.00)	0 (0.00)	1 (2.94)
Blood culture (positive/performed)	64/78	12/24	20/20	32/34
PCR on blood (positive/performed)	13/27	10/13	0/2	3/12
Lumbar puncture performed	44 (56.41)	21 (87.50)	11 (55.00)	12 (35.29)
Confirmed meningitis	10 (12.82)	9 (37.50)	1 (5.00)	0 (0.00)
- Positive culture	2 (2.56)	1 (41.67)	1 (5.00)	0 (0.00)
- Positive PCR	8 (10.26)	8 (33.33)	0 (0.00)	0 (0.00)
Mortality	15 (19.23)	1 (4.17)	2 (10.00)	12 (35.29)

Values are expressed as the number (%)

PCR polymerase chain reaction

The mortality rate was 20% (15 patients) overall, and was especially high in VLBW newborns (12 patients, 35.3% of all VLBW newborns with *E. coli* EOS). Most of the non-survivors had septic shock (14 patients). All had maternal risk factors (93.3% prematurity, 46.6% clinical suspicion of chorioamnionitis, 4% premature rupture of membranes, 33.3% intrapartum maternal fever). All had received ampicillin plus gentamicin as the initial empirical therapy. Cefotaxime had been added in all these neonates because of clinical worsening or antibiogram findings on neonatal or maternal cultures. Mortality rates showed no statistical differences between the periods.

Microbiological data

Fifty-two *E. coli* strains (79%) were resistant to at least one antibiotic. Antibiotic resistance rates over the 4 periods are shown in Table 5. A considerable increase in ampicillin and gentamicin resistance was seen in all the groups, especially along the last 5 years, when ampicillin- and gentamicin-resistant *E. coli* strains reached rates of 92.8 and 28.6%, respectively. A similar increase was observed in the VLBW group. Only gentamicin resistance rates showed a statistically significant difference between periods, probably due to the relatively small sample size. This trend was not observed among VLBW neonates. Of note, none of the strains were resistant to amikacin or carbapenems.

Resistance to third-generation cephalosporins was seen only in the last period (2010–2014). During 2010, 1 extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strain was found in a 28-week-gestation newborn who had septic shock at delivery and died 2 days later in the NICU.

The frequency of resistant *E. coli* strains was the same in the term group and preterm group, including VLBW infants. The presence of these strains was not associated with intrapartum antibiotics or infection during pregnancy. Newborns with resistant *E. coli* EOS showed the same clinical features as those with sensitive *E coli* EOS, and had similar septic shock and mortality rates.

Discussion

This study investigates the epidemiological, clinical, and microbiological data from neonates with *E. coli* EOS in our setting over the last 21 years. During this time, there has been a slight increase in the incidence of *E. coli* EOS, especially from 2005 to 2014, due to a rise in the term and VLBW newborn groups, but it did not reach statistically significance. This result coincides with the findings of studies performed before 2007, which describe a stable overall incidence of *E. coli* regardless of the gestational age and birthweight [1, 3, 4, 13, 20, 27], although 2 studies have reported an increase restricted to VLBW neonates [4, 27]. Antibiotic resistance rates were found to have increased substantially in all our groups, with worrisome resistance to first-line antibiotics, such as ampicillin and gentamicin.

The neonatal and maternal data from our sample were globally comparable to the results from most of the related studies; that is, more than half the affected patients were preterm babies, with a mean birthweight of 1500 to 2000 g [4, 7, 18, 20, 25, 29]. However, Alarcon et al. included more term than preterm newborns [1], and Stoll et al. analyzed only preterm babies with a mean weight of less than 1000 g [27].

Of note, the diagnostic criteria differed between these studies and ours. The definition of *E. coli* EOS was based on *E. coli* detection in blood or CSF culture in different frames of time (from birth to 3, 5, or 7 days of life depending on the study), and none included diagnoses by PCR.

In the last years, we observed an increase in the *E. coli* EOS diagnoses, especially in the term newborn group, due to the systematic use of PCR to determine *E. coli*, *L. monocygotenes* and GBS in neonates with suspicion of EOS (10 newborns with negative cultures were diagnosed by PCR performed in blood or CSF, and 8 of these were term newborns). Naturally, the addition of a complementary exam like PCR to routinely tests would permit to diagnose more EOS than before, so these results must be interpreted with caution. Nonetheless, we are unable to explain why more term neonates tested PCR-positive, when this test was equally used in preterm neonates.

Table 5 Antibiotic resistance in the overall cohort ($n = 65$)

Antibiotic	1994–1999 ($n = 25$)	2000–2004 ($n = 9$)	2005–2009 ($n = 17$)	2010–2014 ($n = 14$)	Total ($n = 65$)	p value
Ampicillin	19 (76)	6 (66.7)	10 (58.8)	13 (92.8)	49 (75.4)	0.153
Gentamicin	*3 (12)*	*0 (0)*	*0 (0)*	*4 (28.6)*	*8 (12.3)*	*0.023*
Amikacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Cefotaxime	0 (0)	0 (0)	0 (0)	1 (7.1)	1 (1.5)	0.105
Imipenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Any antibiotic	21 (84)	7 (77.8)	10 (58.8)	13 (92.8)	51 (78.5)	0.107

Values are expressed as the number (%), p value refers to the difference between periods, MIC breakpoints are the following: ampicillin > 8 mg/L, gentamicin > 4 mg/L, amikacin > 4 mg/L, cefotaxime > 2 mg/L, imipenem > 8 mg/L

With regard to the use of PCR as a diagnostic tool for EOS, Pammi et al. performed a systematic review and metanalysis of 23 studies determining that amplification methods have a sensitivity of 0.90 and a specificity of 0.96 for diagnosis of EOS (using blood culture as gold standard) [22]. These results support the use of amplification assays as an "add-on" test to blood culture, especially in neonates whose mothers have received antibiotics during delivery.

Regarding the role of *E. coli* and GBS as causative agents of EOS, previous studies have shown a predominance of *E. coli* over GBS only in preterm babies, with GBS as the most prevalent pathogen in term babies [18, 25]. In our study, *E. coli* was the leading cause overall and in the preterm and VLBW groups. GBS remained the first pathogen in the term newborn group, although data from the most recent years suggest that *E. coli* EOS is also increasing in this group.

The percentage of resistant *E. coli* strains has increased, particularly those with resistance to ampicillin and gentamicin. This finding is a cause for concern because ampicillin and gentamicin are the recommended first-line antibiotics to be used in newborns with suspected EOS in most guidelines [21]. Many previous publications have alerted to this situation, particularly in relation to the VLBW group [4]. Ampicillin resistance rates higher than 65% have been reported in studies from Europe, America and Asia since the early 2000s [4, 7, 10, 26, 29]. Gentamicin resistance higher than 15% has also been reported [10, 11, 29].

One previous study concluded that intrapartum antibiotic prophylaxis was an independent risk factor for the development of resistance in *E. coli* strains [4], but we did not observe this relationship. A surveillance study in the USA also showed no relationship between ampicillin exposure during delivery and ampicillin-resistant *E. coli* EOS [31]. This is important because an increasingly larger number of newborns are exposed to intrapartum antibiotics (72.2% in our study, similar to reported data [26]).

A global increase in *E. coli* resistance has occurred over recent years, as has been described in the European Center for Disease Control (E-CDC) surveillance report of 2011 [2, 9]. Tameliene et al. investigated maternal and neonatal *E. coli* colonization and found a 21.4% transmission rate, but no relationship between colonization and infection [28]. Additional studies on maternal colonization and perinatal transmission are needed to confirm these findings.

Although we found no relationship between EOS caused by resistant *E. coli* strains and mortality, the high rates of resistance to ampicillin and gentamicin should prompt us to reconsider our empirical therapy, especially in VLBW neonates where *E. coli* EOS is more frequently diagnosed. Moreover, mortality is significantly higher in this group because of their greater immaturity and numerous comorbidities [15, 17]. Other studies have suggested that ampicillin resistance may be related to mortality in *E. coli* EOS [13, 19]. Furthermore, gentamicin may not be a good therapeutic option when the central nervous system (CNS) is affected, a critical consideration in VLBW preterm newborns, who have a particularly high risk of developing meningitis.

Based on these issues, some authors have proposed a third-generation cephalosporin plus ampicillin as empirical EOS therapy [10, 19, 30], whereas others have reported rapid development of resistance when cefotaxime is used routinely for this purpose [5, 12]. In our opinion, this would be a reasonable approach only for selected groups (e.g., VLBW in whom EOS is highly suspected, confirmed maternal chorioamnionitis). Another option would be the use of ampicillin plus amikacin, once CNS involvement has been ruled out.

The limitations of this study are its retrospective nature and the relatively small sample size. Although our sample is the largest reported in our country, the statistical power to detect differences is likely limited. Variations in clinical practice, especially regarding systematic diagnostic PCR use, may have had an effect on the results. Data about antimicrobial susceptibility were not available in cases diagnosed only by PCR.

Furthermore, as our hospital is a referral center, some of the newborns included were born in other centers and referred to our NICU on their first day of life. This may also have occurred in related studies performed in other reference hospitals. A similar number of newborns were referred in each time period, and most were preterm babies. Nonetheless, although they were included for the description of newborn, maternal and microbiological characteristics, we excluded them for the incidence analysis.

To conclude, the predominant role of *E. coli* in EOS in all neonates in our setting and the increasing percentage of resistant strains suggest that a change of empirical treatment may be indicated in the most susceptible newborns. Continuous local and global surveillance is needed to monitor these epidemiological changes and enable adaptation of antimicrobial therapy to the emerging antibiotic susceptibility profiles.

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Authors' contributions AA, AL, MCC and PS conceived and designed the study. MB, NM and SG collected data from electronical and written records. MAF, MCC and NM analyzed and interpreted data. MAF, MB, NM and SG draft the manuscript. AA, AL, MCC and PS reviewed critically the manuscript. All the authors gave final approval of the version to be submitted and any reviewed version.

Compliance with ethical standards The Ethics Committee of HUVH approved the study in May, 2015 (PR(AMI)158/2015). None of the authors received funding for this study. Exemption of informed consent was conceded due to the retrospective character of the study. Patients were codified and no personal information was recorded.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Alarcon A, Pena P, Salas S, Sancha M, Omeñaca F (2004) Neonatal early onset *Escherichia coli* sepsis: trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. Pediatr Infect Dis J 23(4):295–299
2. Allocati N, Masulli M, Alexeyev MF, Di Ilio C (*2013*) *Escherichia coli* in Europe: an overview. Int J Environ Res Public Health *10*(12):6235–6254
3. Andreu A, Ortega E, Planes AM, Salcedo S (2001) Evolution of perinatal Escherichia coli disease in the era of group B Streptococcus prophylaxis. Med Clin (Barc) 117(14):521–524
4. Bizzarro MJ, Dembry LM, Baltimore RS, Gallagher PG (2008) Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics 121(4):689–696
5. Bryan CS, John JF Jr, Pai MS, Austin TL (1985) Gentamicin vs cefotaxime for therapy for early neonatal sepsis. Relationship to drug resistance. Am J Dis Child 139:1086–1089
6. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L (2004) Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 50(2):279–287
7. Cordero L, Rau R, Taylor D, Ayer LW (2004 Jun) Enteric gram-negative bacilli bloodstream infections: 17 years' experience in a neonatal intensive care unit. Am J Infect Control 32(4):189–195
8. Dutta S, Narang A, Chakraborty A, Ray P (2009) Diagnosis of neonatal sepsis using universal primer polymerase chain reaction before and after starting antibiotic drug therapy. Arch Pediatr Adolesc Med 163(1):6–11
9. Gagliotti C, Balode A, Baguero F, Degener J, Grundmann H, Gür D et al. *Escherichia coli* and *Staphylococcus aureus*: bad news and good news from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS), 2002 to 2009. Euro Surveill. 2011;16(11)
10. Guiral E, Bosch J, Vila J, Soto SM (2011) Antimicrobial resistance of *Escherichia coli* strains causing neonatal sepsis between 1998 and 2008. Chemotherapy 58(2):123–128
11. Hasvold J, Bradford L, Nelson C, Harrison C, Attar M, Stillwell T (2013) Gentamicin resistance among *Escherichia coli* strains isolated in neonatal sepsis. J Neonatal Perinatal Med 6(2):137–137
12. Heideking M, Lander F, Hufnagel M, Pfeifer Y, Wicker E, Krause G, Berner R (2013) Antibiotic susceptibility profiles of neonatal invasive isolates of *Escherichia coli* from a 2-year nationwide surveillance study in Germany, 2009-2010. Eur J Clin Microbiol Infect Dis 32:1221–1223
13. Hyde T, Hilger T, Reingold A, Farley MM, O'Brien KL, Schuchat A (2002) Trends in incidence and antimicrobial resistance of early-onset sepsis: population based surveillance in San Francisco and Atlanta. Pediatrics 110(4):690–695
14. Jordan JA, Durso MB (2000) Comparison of 16S rRNA gene PCR and BACTEC 9240 for detection of neonatal bacteremia. J Clin Microbiol 38(7):2574–2578
15. Klein JO, Marcy SM (1995) Bacterial sepsis and meningitis. In: Remington JS, Klein JO (eds) Infectious diseases of the fetus and newborn infant, 4th edn. WB Saunders Co., Philadelphia, pp 835–890
16. Laforgia N, Coppola B, Carbone R, Grassi A, Mautone A, Iolascon A (1997) Rapid detection of neonatal sepsis using polymerase chain reaction. Acta Paediatr 86(10):1097–1099
17. López JB, Coto GD, Fernández B (2005) Neonatal sepsis of vertical transmission: an *epidemiological study from the* "*Grupo de Hospitales Castrillo*". J Perinat Med 28(4):309–315
18. López JB, Fernández B, Coto GD, Ramos A (2005) Grupo de Hospitales Castrillo. Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. Acta Paediatr 94(4):451–457
19. Maayan-Metzger A, Barzialai A, Keller N, Kuint J (2009) Are the 'good old' antibiotics still appropriate for early onset neonatal sepsis? A 10 year survey. Isr Med Assoc J 11:138–142
20. Mayor-Lynn K, González-Quintero VH, O'Sullivan MJ, Harststein AI, Roger S, Tamayo M (2005) Comparison of early-onset neonatal sepsis caused by *Escherichia coli* and *group B Streptococcus*. Am J Obstet Gynecol 192(5):1437–1439
21. Neonatal infection: antibiotics for prevention and treatment. National Institute for Health and Clinical Excellence (NICE) guidelines [CG149]. Published date: August 2012. Last accessed June 2016
22. Pammi M, Flores A, Leeflang M, Versalovic J (2011) Molecular assays in the diagnosis of neonatal sepsis: a systematic review and meta-analysis. Pediatrics 128:973–985
23. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP (1996) Volume of blood required to detect common neonatal pathogens. J Pediatr 129(2):275–278
24. Schrag SJ, Zywicki S, Farley MM, Reingold AL, Harrisson LH, Lefkowitz LB, Hadler JL, Danila R, Cieslak PR, Schuchat A (2000) Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med 342:15–20
25. Stoll BJ, Hansen N, Sánchez PJ, Faix RG, Poindexter BB, Van Meurs PK et al (2011) Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues. Pediatrics 27(5):817–826
26. Stoll BJ, Hansen N (2003) Infections in VLBW infants: studies from the NICHD neonatal research network. Semin Perinatol 27(4):293–301
27. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkraz RA, Lemons JA et al (2002) Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N Engl J Med 347(4): 240–247
28. Tameliene R, Barcaite E, Stoniene D, Buinaskiene J, Markuniene E, Kudreviciene A, Vitkauskiene A, Jomantiene D, Nadisauskiene R (2012) *Escherichia coli* colonization in neonates: prevalence, perinatal transmission, antimicrobial susceptibility, and risk factors. Medicina (Kaunas) 48(2):71–76
29. Tsai C, Chen Y, Wang K, Chen C, Chen C (2012) Characteristics of early-onset neonatal sepsis caused by *Escherichia coli*. Taiwan J Obstet Gynecol 51(1):26–30
30. Wang P (2013) A combination of ampicillin and aminoglycoside for early-onset neonatal sepsis. Editorial on Taiwan. J Obstet Gynecol 52:1–2
31. Weissman SJ, Hansen NI, Zaterka-Baxter K, Higgins RD, Stoll BJ (2015) Emergence of antibiotic resistance-associated clones among *Escherichia coli* recovered from newborns with early-onset sepsis and meningitis in the United States, 2008–2009. J Pediatric Infect Dis Soc