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## Early antibiotic exposure and adverse outcomes in very preterm infants at low risk of early-onset sepsis: the EPIPAGE-2 cohort study

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## ABSTRACT

**Objective:** To assess the association between early empirical antibiotics and neonatal adverse outcomes in very preterm infants without risk factors of early-onset sepsis.

**Study design:** This is a secondary analysis of the EPIPAGE-2 study, a prospective national population-based cohort that included all liveborn infants at 22-31 completed weeks of gestation in France in 2011. Infants at high risk of early-onset sepsis (born after preterm labor or preterm premature rupture of membranes, or from mother who had clinical chorioamnionitis or received antibiotics during the last 72 hours) were excluded. Early antibiotic exposure was defined as antibiotics started at Day 0 or Day 1 of life, regardless of the duration and type of antibiotics. We compared treated and untreated patients by using inverse probability of treatment weighting based on estimated propensity score.

**Results:** Among 648 very preterm infants at low risk of early-onset sepsis, 173 (26.2%) had early antibiotics treatment. Early antibiotic exposure was not associated with death or late-onset sepsis or necrotizing enterocolitis (OR=1.04 [95% CI=0.72-1.50]). However, it was associated with higher odds of severe cerebral lesions (OR=2.71 [95% CI=1.25-5.86]) and moderate-to-severe bronchopulmonary dysplasia (OR=2.30 [95% CI=1.21-4.38]).

**Conclusion:** Early empirical antibiotics administered in very preterm infants at low risk of early-onset sepsis were associated with a higher risk of severe cerebral lesions and moderate-to-severe bronchopulmonary dysplasia.

## INTRODUCTION

Early-onset sepsis (EOS) remains frequent in very preterm infants with a prevalence of 18 per 1000 live births before 28 weeks, which is 10 to 30-fold higher than in term infants.(1) Infection-associated mortality is close to 30% in these vulnerable infants.(1) When facing initial clinical instability in preterm infants, neonatologists are trained to suspect EOS and widely prescribe empirical antibiotic treatments upon admission to neonatal intensive care units (NICU).(2) However, the risk of EOS differs according to the cause of preterm birth. At birth, antibiotic administration is widely performed in the context of preterm labor or preterm premature rupture of membranes due to the high risk of EOS. Nevertheless, these antibiotic treatments are questionable in the context of maternal hypertensive disorders or fetal growth restriction associated with a low risk of EOS.(2,3)

Promoting appropriate use of antibiotics in NICUs is a major concern in neonatology mainly because of their potential adverse impact on antibiotic resistance.(4,5) In addition, antibiotic exposure in early life disrupted the gut microbiota colonization (4,5) and can have long-term effects on health such as obesity, allergic diseases, or inflammatory bowel disease.(6–8)

Studies on early antibiotics treatment in very preterm infants with or without risk factors for EOS have shown that prolonged antibiotic exposure may be associated with neonatal adverse outcomes such as necrotizing enterocolitis (NEC), late-onset sepsis (LOS), bronchopulmonary dysplasia (BPD), and death.(9–12) However, these studies did not usually take into account the cause of preterm birth which is associated with both early antibiotic exposure and some adverse outcomes such as death or LOS.(11,13–16) Moreover, many of these adverse outcomes occur among critically ill preterm infants who received antibiotics due to their severe clinical status at birth. Therefore, controlling for the initial severity of illness would limit the indication bias.(10,11)

To better understand the impact of early antibiotics treatment and reduce the indication bias, one way is to limit the study to very preterm infants at low risk of EOS, namely born after maternal hypertensive disorders and/or fetal growth restriction, and controlling for severity of illness.

The aim of our study was to assess the association between early antibiotic exposure and adverse neonatal outcomes in very preterm infants at low risk for EOS.

## **METHODS**

### **Study population**

This is a secondary analysis of the EPIPAGE-2 cohort (Etude éPIdémiologique sur les Petits Ages GEstationnels 2), a prospective national population-based cohort of preterm infants that included liveborn and stillborn infants as well as all terminations of pregnancy at 22-31 completed weeks of gestation in all maternity units from 25 regions in France in 2011.(17) Participants were recruited over different periods according to gestational age at birth: eight months for births at 22-26 weeks and six months for births at 27-31 weeks. Maternal, obstetric, and neonatal data were collected from medical records following a standardized protocol as previously reported.(18) Recruitment took place at birth after families received information and agreed to participate. During the recruitment period, 8400 births were eligible, among whom 7804 (93%) were enrolled in the study at baseline.(17) EPIPAGE-2 study was approved by the National Data Protection Authority (Commission Nationale de l'Informatique et des Libertés, CNIL n°911009) and by appropriate ethics committees (Consultative Committee on the Treatment of Data on Personal Health for Research Purposes, reference 10.626) and the Committee for the Protection of People participating in biomedical research (reference CPP SC-2873).

All infants enrolled in the EPIPAGE-2 cohort, born alive between 22 and 31 completed weeks were included in this analysis (figure 1). Exclusion criteria were: death in the delivery room or during the first day of life after antenatal decision of withholding care, severe congenital malformation, and twin pregnancies with twin-twin transfusion syndrome. We also excluded infants with a high risk of EOS having an undisputable indication for early antibiotics administration (infants born after preterm labor, preterm premature rupture of membranes or placental abruption, infants whose mother had clinical chorioamnionitis or received antibiotics during the last 72 hours before birth or at birth). Preterm onset of labor and preterm rupture of membranes are considered a clinical indicator of potential intrauterine infection which is associated with a risk of EOS.(3) Very preterm infants born in these contexts were therefore excluded. Clinical chorioamnionitis was defined as maternal fever ( $\geq 37.8^{\circ}\text{C}$ ) associated with at least two of the following criteria: maternal leukocytosis (white blood cell count  $> 15,000$  cells/mm<sup>3</sup>), maternal tachycardia (heart rate  $> 100$  bpm), fetal tachycardia (heart rate  $> 160$  bpm), uterine tenderness and foul-smelling vaginal discharge.(19) Therefore, very preterm infants included in the present study were born after placental-mediated pregnancy complications (hypertensive disorders during pregnancy or fetal growth restriction)(14), and had a very low risk for EOS. Fetal growth restriction was defined by an estimated fetal weight below the 10<sup>th</sup> percentile (according to the reference curves used by the hospital where the antenatal ultrasound scans were performed), in conjunction with growth arrest and/or relevant fetal Doppler abnormalities.

Finally, we excluded infants with missing data for early antibiotics treatment (n=151). Maternal and neonatal characteristics of the study population and the population with missing data for early antibiotics treatment were similar except for the rate of multiple pregnancy which was significantly higher in case of missing data (Table 1 - Online).

## **Main exposure and outcomes**

Early antibiotic exposure was defined as antibiotics started at Day 0 or Day 1 of life, regardless of the duration and type of antibiotics.

Neonatal outcomes included death, LOS, NEC, severe cerebral lesions, and moderate-to-severe BPD during initial hospitalization in NICU. LOS was defined as a positive blood culture after 72 hours of life, associated with antibiotic administration for five days or more, or death within five days following positive blood culture.(20) NEC was defined by the Bell classification of stage  $\geq 2$ .(21) Severe cerebral lesions were defined as intraventricular hemorrhage with ventricular dilatation or parenchymal hemorrhage,(22) or periventricular leukomalacia (i.e., periventricular white matter echolucencies at ultrasonography). Moderate-to-severe BPD was defined as oxygen requirement for at least 28 days and persistent need for oxygen or ventilatory support at 36 weeks' postmenstrual age (mechanical ventilation or positive pressure).(23) Severe neonatal complication was a composite outcome including any of the following: LOS; NEC; severe cerebral lesions; and/or moderate to severe BPD.

We defined the main outcome as a composite outcome accounting for the competing risk between death and adverse outcomes, including at least one of the following: death, LOS and/or NEC. We also considered the following composite outcomes: death or severe neonatal complication, death or severe cerebral lesions, death or moderate-to-severe BPD.

## **Other characteristics considered**

Gestational age at birth was determined as the best obstetrical estimate from early ultrasound assessment and/or last menstrual period.(24) Initial severity of illness was assessed for all infants using the Clinical Risk Index for Babies II (CRIB2) score (25). The CRIB2 score (range 0-27) is based on gestational age, birth weight, neonatal sex, temperature at NICU admission, and initial base deficit. Higher scores of CRIB2 are predictive of higher mortality risk.(25) Newborns were considered small-for-gestational-age if their birth weight was  $\leq 10^{\text{th}}$  percentile



according to French EPOPé intrauterine growth curves adjusted for fetal sex and gestational age (26).

EOS was defined by positive blood or cerebrospinal fluid cultures before 72 hours of life. *Bacillus* and coagulase-negative staphylococci grown on culture of blood <72h were considered contaminants instead of EOS, they were found in 1 and 4 newborns, respectively.(2)

Maternity units were considered as type 3 when associated with a NICU. Inborn status was defined as delivery in a type 3 maternity unit. The volume of the NICU was defined by the number of infants included in the EPIPAGE 2 study in the unit, divided into terciles.

### **Statistical analysis**

We first compared maternal, obstetrical, and neonatal characteristics by early antibiotic treatment (yes vs no). Categorical variables were compared using  $\chi^2$  or Fisher exact tests as appropriate, and medians of quantitative variables by Wilcoxon's test. All descriptive statistics were weighted according to the duration of recruitment. Infants born at 22-26 weeks were recruited over an eight-month period *i.e.* the equivalent of 35 weeks. Infants born at 27-31 weeks were recruited over a period of only six months *i.e.* the equivalent of 26 weeks. We weighted the population of infants born at 27-31 weeks by a factor of 1.34 (35/26) in order to have a population equivalent to what a 35-week recruitment period would have generated. This weighting therefore allowed us to account for the sampling scheme of the cohort and to ensure representativeness.(18) All tests were 2-sided;  $p < 0.05$  was considered statistically significant.

We used a propensity score analysis to minimize the indication bias in estimating the antibiotic exposure effect.(27) The first step in the analysis consisted of estimating the propensity score, defined as the newborn's probability of receiving early antibiotic treatment based on his/her individual observed characteristics at admission to NICU. The score was estimated using a logistic regression model with the administration of early antibiotic treatment as the dependent variable, regressed on baseline characteristics clinically associated with the

exposure and/or the outcome, excluding covariates that might be a consequence of the treatment.(28) We included the following covariates: maternal age (<25, 25-34 or ≥35 years) (29), maternal place of birth (30), overweight (body mass index (BMI) ≥ 25) (31), smoking status during pregnancy (32), antenatal corticosteroids administration (at least one injection administered before delivery), single or multiple pregnancy, inborn status, gestational age at birth, birth weight (<1000, 1000-1499, or ≥1500 grams), neonatal sex, CRIB2 score, intubation in the delivery room, surfactant administration in the delivery room and NICU characteristics (level and volume of activity of the unit where the infant was hospitalized during the first 48 hours of life.).

The second step in the analysis involved inverse probability of treatment weighting (IPTW). IPTW was based on estimated propensity scores, to obtain a synthetic sample (IPTW sample), in which antibiotic treatment assignment was independent of measured baseline covariates.(27,33,34) Balance in the observed baseline covariates between treated and untreated patients in the initial and IPTW samples was assessed by examining standardized differences (Figure 2 - Online). Finally, we estimated the association between early antibiotics exposure and outcomes by a multilevel logistic regression within the weighted sample, with NICU as level 2 to account for the wide variation in the proportion of newborns that received early antibiotic treatment by center (Figure 3 - Online). We adjusted for gestational age because it was considered a confounding factor (a common ancestor of both the exposure and outcomes), and was not perfectly balanced in the propensity score (Figure 2 - Online). Results were presented as ORs with a 95% confidence interval (95%CI).

We performed a sensitivity analysis after multiple imputations for missing data. The proportion of missing data ranged from 0.0% to 8.9% for each covariate, and missing data were considered missing at random. Multiple imputations were used including all baseline variables and outcomes of the propensity-score model. Categorical variables were imputed using logistic or

multinomial regression as appropriate. We generated 50 independent imputed datasets. A propensity score was estimated for each of the 50 imputed data sets generated, and results were pooled for a final analysis according to Rubin's rules.(35)

All statistical analyses were performed with STATA statistical software, version 16.1 (StataCorp).

## **RESULTS**

We included 648 very preterm infants at low risk for EOS among which 173 (26.2%) received early antibiotic treatment started at Day 0 or Day 1 of life (figure 1).

Maternal, obstetrical, and neonatal characteristics are presented in Table 2. In this sample of pregnant women with hypertensive disorders and/or with fetal growth restriction, median maternal age was 30 years (interquartile range [IQR] 26-35), 45.5% were overweight at the beginning of pregnancy, 58.5% primipara and 20.3% smoked during pregnancy. Antenatal corticosteroids were widely administered, almost all babies were delivered by cesarean section. Infants exposed to early antibiotics were more frequently born from multiple pregnancies but less frequently in a level 3 maternity unit. Other maternal and obstetrical characteristics did not differ by early exposure to antibiotics.

The median gestational age at birth was 30.0 weeks (IQR 28.4-31.0) and the median birth weight was 1040 grams (IQR=820-1260). Infants who received early antibiotic treatment had a slightly lower median gestational age at birth (29.6 versus 30.3 weeks) and lower median birth weight (1010 versus 1060 grams). Delivery room intubation and surfactant administration were more frequent in the group of infants with early antibiotics. However, the severity of the initial illness, measured by the CRIB2 score at admission in NICU did not differ significantly (Table 2).

Among the very preterm infants who received early antibiotics, 88.1% had treatment initiated at Day 0. Details about antibiotic treatment received were available for 165 patients. All children received a combination of at least 2 molecules. Amoxicillin was used in 112/165 patients, third-generation cephalosporin in 129/165 patients, and aminoglycosides in 140/165 patients. Vancomycin was also used in combination in 11 patients, and metronidazole in 5. The median duration of antibiotic administration was 3 days (IQR=2-6).

Four infants presented an episode of EOS diagnosed at the second or third day of life. None had received antibiotic treatment within the first day of life. Identified bacteria were *Streptococcus agalactiae* in one case and *Staphylococcus aureus* in the three others (including one child who died on Day 3).

Neonatal outcomes and their association with early exposure to antibiotics are described in table 3. After IPTW, standardized differences between treated and untreated patients were below 0.10 for all covariates, except for a few gestational age groups (Figure 2 - Online).

Mortality was more frequent in the early exposure to antibiotics group (12.0% versus 6.3%). The frequency of NEC was similar in both groups (5.9% in the early antibiotics group vs 4.9%). In our population, 25.3% presented at least one episode of LOS at a median age of 12 days (IQR=7-20), with no difference between the two groups. There were 165 episodes of LOS including 41 in the group with early exposure to antibiotics and 124 in the group without exposure to antibiotics. The most common pathogens found in blood cultures collected during LOS were coagulase-negative *staphylococci* (75.5%), *Staphylococcus aureus* (15.7%), *Enterococcus sp.* (3.1%), and Gram-negative organisms (2.6%), with no difference between the two groups. We did not identify any episode of LOS due to a bacterium resistant to the initial early antibiotics administered. Late-onset sepsis characteristics did not differ according to early neonatal antibiotics (Table 4 – online). In the complete cases analyses, we did not find any association between early exposure to antibiotics and death (OR=1.78 [95% CI=0.82-3.88]),

LOS (OR=0.93 [95%CI=0.64-1.34]) or NEC (OR=1.47 [95%CI=0.65-3.33]). Results were consistent after multiple imputations. The composite outcome (death or LOS or NEC) did not significantly differ between the two groups (35.8% vs 32.5%) and we did not find an association between early exposure to antibiotics and this composite outcome (OR=1.04 [95%CI=0.72-1.50]).

However, severe cerebral lesions, isolated or combined with death, were more frequent when newborns received early antibiotics, (9.1% vs 4.5%, and 16.6% vs 9.9%, respectively). We found an association between severe cerebral lesions and early exposure to antibiotics (OR=2.71 [95%CI=1.25-5.86]). The trend was the same, but at the limit of significance (OR=2.99 [95%CI=0.99-9.04]), after multiple imputations. The associations were less pronounced for the composite outcome death or severe cerebral lesions (Table 3).

Moderate-to-severe BPD was also more frequent in the early exposure to antibiotics group, whether isolated or associated with death (19.7% vs 10.2% and 28.6% vs 15.4%, respectively). We found an association between moderate-to-severe BPD and early exposure to antibiotics (OR=2.30 [95%CI=1.21-4.38]). The trend was the same, but at the limit of significance (OR=1.87 [95%CI=0.96-3.65]), after multiple imputations. Results were similar for the composite outcome death or moderate-to-severe BPD (Table 3).

Finally, we studied the composite outcome “severe neonatal complication” (which included any of the following: LOS; NEC; severe cerebral lesions; and/or moderate to severe BPD), and did not find an association with early exposure to antibiotics (OR=1.37 [95%CI=0.94-1.98]).

In a sensitivity analysis after multiple imputations for missing data, results were consistent for the composite outcome (death or LOS or NEC) (Table 5). For severe cerebral lesions and severe BPD, the trend was the same than complete cases analysis, but at the limit of significance (OR=2.99 [95%CI=0.99-9.04] and OR=1.87 [95%CI=0.96-3.65], respectively).

## DISCUSSION

Among the 648 very preterm infants at low risk for EOS, 26% were exposed to antibiotics during the first day of life. This early antibiotic exposure was not associated with death, LOS and/or NEC, but was associated with a higher risk of severe cerebral lesions and moderate-to-severe BPD.

The strengths of our study included the use of data from a national prospective population-based cohort study. Data about antibiotic exposure and neonatal outcomes were prospectively collected using a detailed and standardized questionnaire. We had a relatively small sample, but our study provided a unique opportunity to evaluate the impact of antibiotic prescription on neonatal outcomes, independently of indication biases: firstly, we chose to study a homogeneous sample of very preterm infants at low risk of EOS. We therefore excluded those born following: spontaneous preterm labor, rupture of membranes, maternal antibiotic treatment during the last 72 hours before birth, placental abruption or clinical chorioamnionitis. These infants had none of the EOS risk factors that would require empirical antibiotic treatment at birth, and only four (in the group without initial exposition to antibiotics) were finally diagnosed with EOS. Among them, three infants were infected with *S. aureus*. In one case, the infection started on day 2, and in the two other cases on day 3. In this context of preterm birth with cesarean section, vertical transmission is very unlikely and these 3 infections were probably hospital-acquired infection.

Altogether, this empirical administration of antibiotics that involved 26% of our population was probably started out of standard protocols, with wide variations between NICUs. This rate was consistent with previous reports and should be addressed with specific antibiotic stewardship interventions.(2,11) Then, we controlled for the initial severity of illness including the CRIB2 score in the propensity score, a validated predictor of mortality in this population.(25) We also added intubation and surfactant administration in the delivery room to account for clinical

severity at birth. The initial clinical severity is a confounding factor, associated with both early antibiotics treatment (empirical treatment of possible sepsis) and neonatal adverse outcomes.(2) Finally, to limit the effect of the indication bias, we used a propensity score, based on variables available at the time of decision to initiate or not an early antibiotic treatment. Using IPTW enabled us to estimate the average treatment effect on the whole sample.(34) In contrast, using the matching on propensity score, patients for whom no match existed are excluded, which results in a significant loss of information that ultimately leads to a selection bias, in particular in this population of infants with low risk of EOS at baseline. It provided a way to balance measured covariates across exposed and non-exposed groups independently of the clinical status at admission in NICU that may have influenced the decision to administrate early antibiotics treatment.

Limitations should be kept in mind. We had a limited sample size due to our choice of a low risk of EOS study population. As a part of this epidemiological study, we could not analyze the gut microbiota nor the impact of early antibiotic exposure on subsequent colonization with multi-drug resistant organisms.

In our study, early antibiotic exposure was not associated with a higher risk of death, LOS, and/or NEC. Several studies found an association between early empirical exposition to antibiotics and these outcomes, isolated or combined.(9–11) However, these studies have included preterm infants alive at day 5 (9), at day 7 (11) or day 14 (10), to have sufficient time to be exposed to antibiotics and focused on consequences of duration of antibiotics treatment. We chose to study mortality and morbidities from the first day of life to obtain a complete assessment of the putative consequences of early antibiotics exposure. In a subgroup of very low birthweight infants with low risk of EOS, Ting *et al* found an increased risk of a composite outcome (intraventricular hemorrhage, NEC, BPD, severe retinopathy of prematurity, hospital-acquired infections, or death) in case of long antibiotic exposure (4-7 days) versus none

(aOR=1.51 [95%CI=1.22-1.87]) but no statistically significant difference in case of short antibiotic exposure (1-3 days) (aOR=1.03 [95%CI=0.84-1.26]). (11) More recently, Li *et al* found a lower incidence of NEC in case of early antibiotic administration in an international cohort of 2831 preterm very low birthweight infants (aOR=0.25 [95%CI=0.12-0.47]).(16) In this study, almost 90% of infants received antibiotics within the first three days after birth. Moreover, in the small comparison group that did not receive early antibiotics treatment (269/2831), baseline characteristics differed markedly such as higher gestational age, higher rate of cesarean delivery, and higher proportion of small-for-gestational-age infants. The relatively high NEC incidence in this control group could lead to a potential confounding bias and requires caution in interpretation.

In our study, early antibiotic exposure was associated with a higher risk of moderate-to-severe BPD and severe cerebral lesions, despite adjustment for the severity of initial illness. Our findings were similar to those reported in some studies.(11,12) Puopolo *et al* find also similar results: among 5334 low-EOS risk extremely preterm infants, those empirically treated with early prolonged antibiotics in the absence of culture-confirmed infection had a higher adjusted risk of severe cerebral lesions and higher adjusted risk of BPD compared to the infants who were not given early prolonged antibiotics (adjusted for multiple predictors of illness severity).(2)

We cannot totally rule out a lack of power to explain our observed lack of associations between early antibiotics and death, LOS, and/or NEC. Nevertheless, concerning the association between early antibiotics and severe cerebral lesions and bronchopulmonary dysplasia that we have found, the pathophysiological mechanisms remain to be understood. Consequences of early antibiotics should be different according to the type of antibiotics used or the number of courses during the hospitalization for example. Studying the consequences of early antibiotic treatment in very preterm infants is complex, and requires assessing not only neonatal mortality



and morbidity but also effects on microbiota and later health. Indeed, postnatal exposure to antibiotics has been shown to impact the diversity of the gut microbiota.(4) Other concerns also include a potential impact on host immunity and subsequent auto-inflammatory disorders, according to the suggested consequence of microbiota on inflammation and in the pathogenesis of a wide variety of inflammatory disease processes.(36) Tracheal microbiota seems also to be different between infants who develop bronchopulmonary dysplasia and those who do not and postnatal exposition to antibiotics could be involved in these differences.(36)

In order to reach a more robust conclusion on the consequences of early antibiotics in preterm infants, a randomized controlled trial would be optimal. This study should include very preterm infants, born between 26 and 31 weeks, without risk factor for early-onset sepsis and the primary outcome will be the composite outcome of LOS, NEC, severe cerebral lesions and BPD or death.

## **CONCLUSION**

Early exposure to antibiotics was not associated with an increased risk of a composite outcome including death, NEC and/or LOS in very preterm infants at low risk for EOS. However, our results question whether exposure to early antibiotics is warranted since it was associated with a higher risk of severe cerebral lesions and moderate-to-severe BPD.

## **Abbreviations**

BPD bronchopulmonary dysplasia, EOS Early-onset sepsis, IPTW inverse probability of treatment weighting, LOS Late-onset sepsis, NEC necrotizing enterocolitis, NICU Neonatal intensive care unit

## **CONTRIBUTORS**

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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## REFERENCES

1. Stoll BJ, Puopolo KM, Hansen NI, Sánchez PJ, Bell EF, Carlo WA, et al. Early-Onset Neonatal Sepsis 2015 to 2017, the Rise of *Escherichia coli*, and the Need for Novel Prevention Strategies. *JAMA Pediatr.* 2020;174:e200593.
2. Puopolo KM, Mukhopadhyay S, Hansen NI, Cotten CM, Stoll BJ, Sanchez PJ, et al. Identification of Extremely Premature Infants at Low Risk for Early-Onset Sepsis. *Pediatrics.* 2017;140:e20170925.
3. Benitz WE, Gould JB, Druzin ML. Risk factors for early-onset group B streptococcal sepsis: estimation of odds ratios by critical literature review. *Pediatrics.* 1999;103:e77.
4. Arboleya S, Sánchez B, Milani C, Duranti S, Solís G, Fernández N, et al. Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. *J Pediatr.* 2015;166:538–44.
5. de Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. *Lancet.* 2000;355:973–8.
6. Bailey LC, Forrest CB, Zhang P, Richards TM, Livshits A, DeRusso PA. Association of antibiotics in infancy with early childhood obesity. *JAMA Pediatr.* 2014;168:1063–9.
7. Hoskin-Parr L, Teyhan A, Blocker A, Henderson AJW. Antibiotic exposure in the first two years of life and development of asthma and other allergic diseases by 7.5 yr: a dose-dependent relationship. *Pediatr Allergy Immunol.* 2013;24:762–71.
8. Kronman MP, Zaoutis TE, Haynes K, Feng R, Coffin SE. Antibiotic exposure and IBD development among children: a population-based cohort study. *Pediatrics.* 2012;130:e794-803.
9. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics.* 2009;123:58–66.
10. Cantey JB, Pyle AK, Wozniak PS, Hynan LS, Sánchez PJ. Early Antibiotic Exposure

and Adverse Outcomes in Preterm, Very Low Birth Weight Infants. *J Pediatr.* 2018;203:62–7.

11. Ting JY, Roberts A, Sherlock R, Ojah C, Cieslak Z, Dunn M, et al. Duration of Initial Empirical Antibiotic Therapy and Outcomes in Very Low Birth Weight Infants. *Pediatrics.* 2019;143:e20182286.

12. Canteley JB, Huffman LW, Subramanian A, Marshall AS, Ballard AR, Lefevre C, et al. Antibiotic Exposure and Risk for Death or Bronchopulmonary Dysplasia in Very Low Birth Weight Infants. *J Pediatr.* 2017;181:289-293.e1.

13. Esaiassen E, Fjalstad JW, Juvet LK, van den Anker JN, Klingenberg C. Antibiotic exposure in neonates and early adverse outcomes: a systematic review and meta-analysis. *J Antimicrob Chemother.* 2017;72:1858–70.

14. Delorme P, Goffinet F, Ancel P-Y, Foix-L'Hélias L, Langer B, Lebeaux C, et al. Cause of Preterm Birth as a Prognostic Factor for Mortality. *Obstet Gynecol.* 2016;127:40–8.

15. Letouzey M, Foix-L'Hélias L, Torchin H, Mitha A, Morgan AS, Zeitlin J, et al. Cause of preterm birth and late-onset sepsis in very preterm infants: the EPIPAGE-2 cohort study. *Pediatr Res.* 2021; 24:1–9.

16. Li Y, Shen RL, Ayede AI, Berrington J, Bloomfield FH, Busari OO, et al. Early Use of Antibiotics Is Associated with a Lower Incidence of Necrotizing Enterocolitis in Preterm, Very Low Birth Weight Infants: The NEOMUNE-NeoNutriNet Cohort Study. *J Pediatr.* 2020;227:128-134.e2.

17. Lorthe E, Benhammou V, Marchand-Martin L, Pierrat V, Lebeaux C, Durox M, et al. Cohort Profile: The Etude Epidémiologique sur les Petits Ages Gestationnels-2 (EPIPAGE-2) preterm birth cohort. *Int J Epidemiol.* 2021:1-15

18. Ancel P-Y, Goffinet F, EPIPAGE 2 Writing Group. EPIPAGE 2: a preterm birth cohort in France in 2011. *BMC Pediatrics.* 2014;14:97.

19. Gibbs RS. Diagnosis of intra-amniotic infection. *Semin Perinatol.* 1977;1:71–7.

20. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics*. 2002;110:285–91.
21. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*. 1978;187:1–7.
22. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol*. 2009;8:110–24.
23. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*. 2001;163:1723–9.
24. Ancel P-Y, Goffinet F, EPIPAGE-2 Writing Group. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study. *JAMA Pediatr*. 2015;169:230–8.
25. Parry G, Tucker J, Tarnow-Mordi W, UK Neonatal Staffing Study Collaborative Group. CRIB II: an update of the clinical risk index for babies score. *Lancet*. 2003;361:1789–91.
26. Ego A, Prunet C, Lebreton E, Blondel B, Kaminski M, Goffinet F, et al. [Customized and non-customized French intrauterine growth curves. I - Methodology]. *J Gynecol Obstet Biol Reprod*. 2016;45:155–64.
27. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivar Behav Res*. 2011;46:399–424.
28. Garrido MM, Kelley AS, Paris J, Roza K, Meier DE, Morrison RS, et al. Methods for Constructing and Assessing Propensity Scores. *Health Serv Res*. 2014;49:1701–20.
29. Klemetti R, Gissler M, Sainio S, Hemminki E. At what age does the risk for adverse maternal and infant outcomes increase? Nationwide register-based study on first births in Finland in 2005-2014. *Acta Obstet Gynecol Scand*. 2016;95:1368–75.

30. Howell EA, Janevic T, Hebert PL, Egorova NN, Balbierz A, Zeitlin J. Differences in Morbidity and Mortality Rates in Black, White, and Hispanic Very Preterm Infants Among New York City Hospitals. *JAMA Pediatr.* 2018;172:269–77.
31. Declercq E, MacDorman M, Cabral H, Stotland N. Prepregnancy Body Mass Index and Infant Mortality in 38 U.S. States, 2012-2013. *Obstet Gynecol.* 2016;127:279–87.
32. Dietz PM, England LJ, Shapiro-Mendoza CK, Tong VT, Farr SL, Callaghan WM. Infant morbidity and mortality attributable to prenatal smoking in the U.S. *Am J Prev Med.* 2010;39:45–52.
33. Thomas L, Li F, Pencina M. Using Propensity Score Methods to Create Target Populations in Observational Clinical Research. *JAMA.* 2020;323:466–7.
34. Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. *Stat Med.* 2015;34:3661–79.
35. Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. *Stat Med.* 1991;10:585–98.
36. Underwood MA, Sohn K. The Microbiota of the Extremely Preterm Infant. *Clin Perinatol.* 2017;44:407–27.

## FIGURE LEGENDS

Figure 1: Flow chart

Figure 2 (online): Standardized differences in the initial and IPTW samples in complete-cases (A) and after multiple imputation (B)

Each covariate included in the propensity score is on the y axis.

Figure 3 (online): Variations between NICUs in the proportion of very preterm infants at low risk for early-onset sepsis treated with early antibiotics: number of infants admitted to each NICU (blue bars, from 1 to 24) and proportion (%) of infants treated with early antibiotics (orange solid line, from 0 to 100%).

**Table 1 (online):** Comparison of maternal and obstetrical characteristics between the study population and the population with missing data for early neonatal antibiotics

	Total N=799	Early neonatal antibiotics		p
		Complete cases N=648 (80.9%)	Missing data N=151 (19.1%)	
<b>Maternal characteristics</b>				
Mother's age (N=799) median (IQR)	30 (26-35)	30 (26-35)	30 (26-33)	0.95
Body mass index $\geq$ 25 (N=740)	343 (46.3)	274 (45.5)	69 (49.6)	0.39
Mother's place of birth (N=790)				0.07
Europe	595 (75.3)	474 (73.9)	121 (81.2)	
Northern Africa	70 (8.9)	55 (8.6)	15 (10.2)	
Subsaharian Africa	77 (9.9)	69 (10.9)	8 (5.4)	
Other	48 (5.9)	43 (6.6)	5 (3.2)	
Primiparous (N=791)	467 (59.0)	375 (58.5)	92 (61.0)	0.19
Smoking during pregnancy (N=767)	151 (19.8)	125 (20.3)	26 (17.8)	0.50
Multiple pregnancy (N=799)	131 (16.5)	83 (12.8)	48 (32.0)	<0.001
Antenatal corticosteroids (N=799)	702 (87.7)	571 (88.3)	131 (85.6)	0.38
Cesarean section (N=799)	801 (99.4)	647 (99.4)	154 (99.4)	0.98
<b>Neonatal characteristics</b>				
Gestational age (weeks) median (IQR) (N=799)	30 (28-31)	30 (28-31)	30 (28-31)	0.93
Male sex (N=799)	375 (47.2)	304 (47.1)	71 (47.3)	0.96
Birth weight (grams) median (IQR) (N=799)	1040 (830-1255)	1040 (820-1260)	1050 (880-1250)	0.79
CRIB2 score (N=678)	6 (4-9)	6 (4-9)	6 (5-8)	0.91
Intubation in delivery room (N=775)	396 (50.1)	329 (51.5)	67 (44.4)	0.12
Early-onset sepsis (N=798)	5 (0.6)	4 (0.6)	1 (0.7)	0.96
Death (N=799)	66 (7.6)	55 (7.8)	11 (6.8)	0.62
Late-onset sepsis (N=783)	199 (25.0)	165 (25.4)	34 (23.2)	0.59
Necrotizing enterocolitis (N=786)	38 (4.8)	33 (5.2)	5 (3.5)	0.40
Severe cerebral lesions (N=784)	44 (5.4)	38 (5.7)	6 (4.1)	0.43
Bronchopulmonary dysplasia (N=719)	99 (13.0)	78 (12.6)	21 (15.2)	0.41

IQR interquartile range



**Table 2:** Maternal, obstetrical, and neonatal characteristics according to early neonatal antibiotics administration in very preterm infants

	Total N=648	Early neonatal antibiotics		p
		No N=475 (73.8%)	Yes N=173 (26.2%)	
<b>Maternal characteristics</b>				
Mother's age (N=648)				0.50
< 25 years	117 (18.1)	87 (18.4)	30 (17.2)	
25-34 years	364 (56.0)	262 (55.0)	102 (58.9)	
≥ 35 years	167 (25.9)	126 (26.5)	41 (24.0)	
BMI ≥ 25 (N=601)	274 (45.5)	206 (46.6)	68 (42.5)	0.38
Mother's place of birth (N=641)				0.58
Europe	474 (73.9)	346 (73.8)	128 (74.3)	
Northern Africa	55 (8.6)	41 (8.7)	14 (8.3)	
Subsaharian Africa	69 (10.9)	54 (11.6)	15 (8.9)	
Other	43 (6.6)	28 (5.9)	15 (8.5)	
Primiparous (N=640)	375 (58.5)	278 (59.1)	97 (56.9)	0.74
Smoking during pregnancy (N=621)	125 (20.3)	91 (19.9)	34 (21.6)	0.65
Multiple pregnancy (N=648)	87 (12.9)	51 (10.9)	32 (17.5)	0.006
Antenatal corticosteroids* (N=639)	561 (88.1)	419 (89.3)	142 (84.4)	0.09
Cesarean section (N=641)	637 (99.4)	466 (99.6)	171 (98.8)	0.29
Inborn** (N=648)	600 (92.7)	449 (94.6)	151 (87.4)	0.002
<b>Neonatal characteristics</b>				
Gestational age (weeks) (N=648)				0.09
25-27	127 (17.6)	84 (16.1)	43 (21.6)	
28-29	196 (31.0)	140 (30.0)	56 (33.8)	
30-31	325 (51.4)	251 (53.8)	74 (44.6)	
Male sex (N=648)	304 (47.1)	216 (45.6)	88 (51.5)	0.18
Birth weight (grams) (N=648)				0.14
<1000	286 (42.7)	199 (40.8)	87 (48.1)	
1000-1499	316 (50.0)	244 (52.3)	72 (43.4)	
≥1500	46 (7.3)	32 (6.9)	14 (8.4)	
SGA (<10th) (N=648)	521 (80.2)	386 (81.1)	135 (77.7)	0.34
Apgar score <5 at 5' (N=625)	54 (8.5)	34 (7.2)	20 (12.4)	0.04
Intubation in delivery room (N=626)	329 (51.5)	213 (45.8)	116 (67.3)	<0.001
Surfactant in delivery room (N=635)	195 (29.9)	132 (27.7)	63 (36.1)	0.04
Chest compressions in delivery room (N=617)	28 (4.3)	18 (3.8)	10 (5.7)	0.29
CRIB2 (N=555)	6 (4.9)	6 (4.9)	7 (4.9)	0.18
Early-onset sepsis (N=648)	4 (0.6)	4 (0.9)	0 (0.0)	0.23
<b>Neonatal unit characteristics ***</b>				
Level 3 (N=648)	629 (97.0)	463 (97.4)	166 (95.8)	0.29
Volume of activity (N=648)				0.13
Low	203 (31.6)	145 (30.7)	58 (34.1)	
Medium	234 (35.9)	165 (34.5)	69 (39.8)	
High	211 (32.6)	165 (34.8)	46 (26.2)	

Data are presented as number (percentage) of patients. Percentages are weighted according to the differences between length of recruitment of gestational age groups.

For each variable, percentages might not sum up to 100%, owing to rounding.

IQR interquartile range; SGA small-for-gestational-age; CRIB Clinical risk index for babies score

\* Antenatal corticosteroids were defined as at least one injection of corticosteroids before delivery

\*\* Inborn was defined as birth in a hospital with a neonatal intensive care unit

\*\*\* Characteristics of the unit in which the newborn was hospitalized during the first 48 hours of life.

**Table 3:** Neonatal outcomes according to early neonatal antibiotics

	Total	Early neonatal antibiotics		p	Complete-cases analysis (N=465) aOR (95% CI)
	N=648	No N=475 (73.8)	Yes N=173 (26.2)		
Death (N=648)	55 (7.8)	32 (6.3)	23 (12.0)	0.01	1.78 (0.82-3.88)
LOS (N=641)	165 (25.4)	124 (26.0)	41 (23.3)	0.35	0.93 (0.64-1.34)
NEC (N=641)	33 (5.2)	23 (4.9)	10 (5.9)	0.62	1.47 (0.65-3.33)
Severe cerebral lesions (N=635)	38 (5.7)	21 (4.5)	17 (9.1)	0.02	2.71 (1.25-5.86)
Moderate to severe BPD (N=585)	78 (12.6)	47 (10.2)	31 (19.7)	<0.01	2.30 (1.21-4.38)
<b>Composite outcomes</b>					
Severe neonatal complication* (N=604)	252 (40.8)	174 (38.6)	78 (46.9)	0.07	1.37 (0.94-1.98)
Death or severe neonatal complication (N=647)	274 (41.3)	188 (38.9)	86 (48.0)	0.04	1.33 (0.92-1.93)
Death or severe cerebral lesions (N=640)	79 (11.7)	48 (9.9)	31 (16.6)	0.02	2.16 (1.21-3.85)
Death or moderate to severe BPD (N=634)	128 (18.9)	76 (15.4)	52 (28.6)	<0.001	2.25 (1.31-3.87)
Death or LOS or NEC (N=639)	218 (33.3)	154 (32.5)	64 (35.8)	0.43	1.04 (0.72-1.50)

Data are presented as number (percentage) of patients. Percentages are weighted according to the differences between length of recruitment of gestational age groups.

For each variable, percentages might not sum up to 100%, owing to rounding.

LOS Late-onset sepsis; NEC necrotizing enterocolitis; BPD bronchopulmonary dysplasia; aOR adjusted odd ratio

Adjusted odds ratio (aOR) obtained from multilevel analysis with inverse probability of treatment weighting, adjusted for gestational age.

\*Severe neonatal complication was defined as any of the following outcomes: late-onset sepsis; severe cerebral lesions (intraventricular hemorrhage with ventricular dilatation, parenchymal hemorrhage, and/or cystic periventricular leukomalacia); Bell's stage  $\geq 2$  necrotizing enterocolitis; and/or moderate-to-severe bronchopulmonary dysplasia (BPD), defined as administration of oxygen for at least 28 days plus need for 21% or more oxygen and/or mechanical ventilatory support or continuous positive airway pressure at 36 weeks' postmenstrual age.

**Table 4 (online):** Late-onset sepsis characteristics according to early neonatal antibiotics

	<b>Total</b>	<b>Early neonatal antibiotics</b>		<b>p</b>
	<b>N=648</b>	<b>No N=475 (73.8)</b>	<b>Yes N=173 (26.2)</b>	
LOS (N=641)				0.35
0	476 (74.6)	347 (74.0)	129 (76.7)	
1 episode	141 (21.7)	109 (22.9)	32 (18.6)	
2 or 3 episodes	24 (3.6)	15 (3.2)	9 (4.7)	
Age at 1st episode of LOS (days) median (IQR) (N=164)	12 (7-20)	11 (7-18)	12 (8-20)	0.86
Pathogens implicated in LOS				0.75
coagulase-negative <i>staphylococci</i>	120 (75.5)	90 (75.6)	30 (75.0)	
<i>Staphylococcus aureus</i>	25 (15.7)	20 (16.6)	5 (12.9)	
<i>Enterococcus sp.</i>	5 (3.1)	3 (2.6)	2 (4.7)	
Gram-negative organisms	4 (2.6)	2 (1.7)	2 (5.4)	
Others	4 (2.5)	3 (2.6)	1 (2.0)	

Data are presented as number (percentage) of patients unless otherwise indicated. Percentages are weighted according to the differences between length of recruitment of gestational age groups.

For each variable, percentages might not sum up to 100%, owing to rounding.

LOS Late-onset sepsis; IQR interquartile range;

**Table 5:** Sensitivity analysis: Association between neonatal outcomes and early neonatal antibiotics after multiple imputation.

	Complete-cases analysis (N=465) aOR (95% CI)	Multiple imputation (N=648) aOR (95% CI)
Death	1.78 (0.82-3.88)	1.51 (0.57-3.99)
LOS	0.93 (0.64-1.34)	1.12 (0.62-2.03)
NEC	1.47 (0.65-3.33)	*
Severe cerebral lesions	2.71 (1.25-5.86)	2.99 (0.99-9.04)
Moderate-to-severe BPD	2.30 (1.21-4.38)	1.87 (0.96-3.65)
<b>Composite outcomes</b>		
Any severe neonatal complication	1.37 (0.94-1.98)	1.54 (0.87-2.72)
Death or severe neonatal complication	1.33 (0.92-1.93)	1.53 (0.85-2.73)
Death or severe cerebral lesions	2.16 (1.21-3.85)	1.85 (0.82-4.18)
Death or moderate-to-severe BPD	2.25 (1.31-3.87)	1.76 (0.91-3.39)
Death or LOS or NEC	1.04 (0.72-1.50)	1.28 (0.72-2.28)

Adjusted odds ratio (aOR) obtained from multilevel analysis with inverse probability of treatment weighting, adjusted for gestational age.

OR Odds ratio; LOS late-onset sepsis, NEC necrotizing enterocolitis, BPD bronchopulmonary dysplasia. Severe neonatal complication was defined as any of the following outcomes: late-onset sepsis; severe cerebral lesions (intraventricular hemorrhage with ventricular dilatation, parenchymal hemorrhage, and/or cystic periventricular leukomalacia); Bell's stage  $\geq 2$  necrotizing enterocolitis; and/or moderate-to-severe BPD

\* Convergence not achieved due to the low number of cases of NEC.

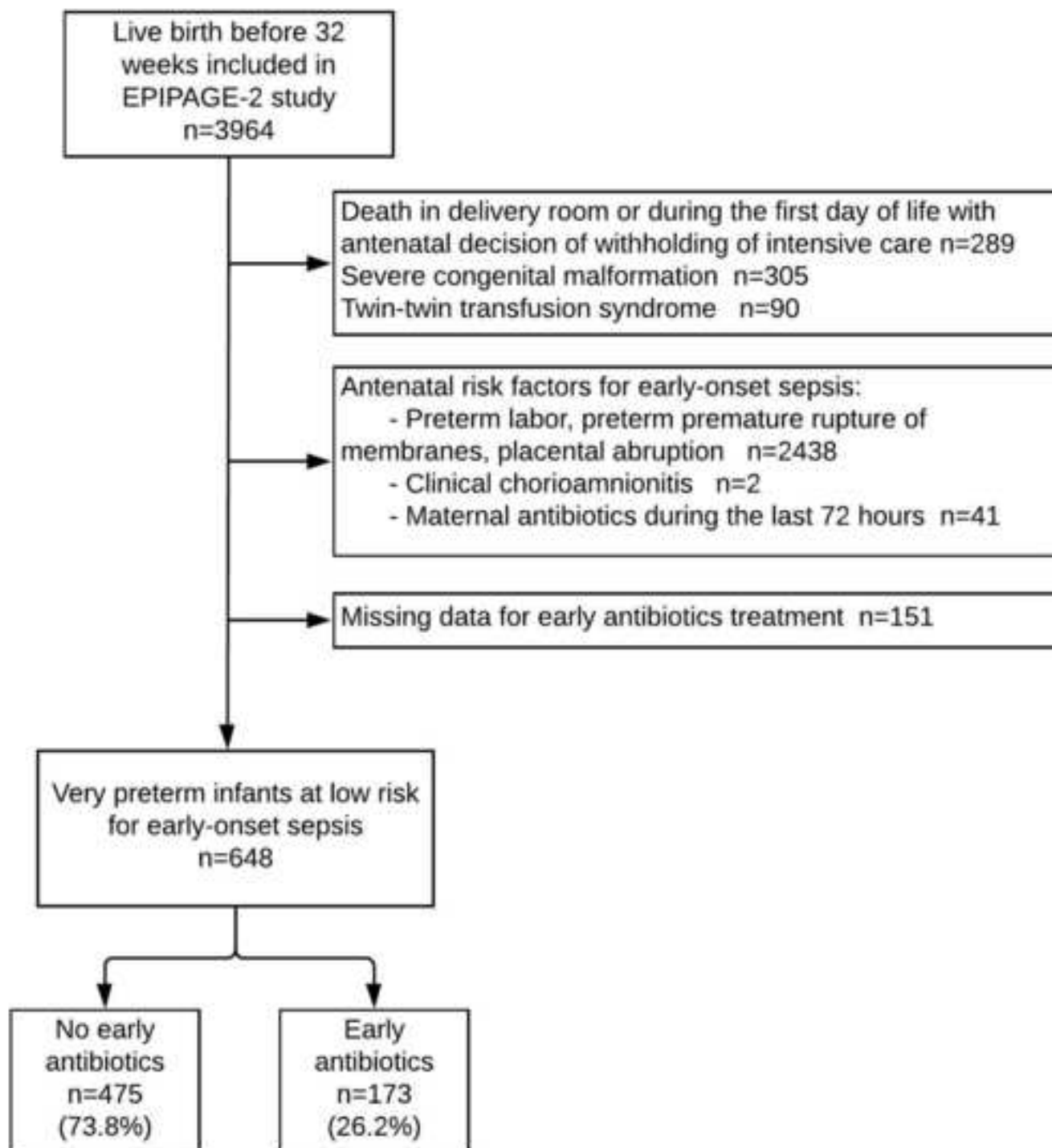
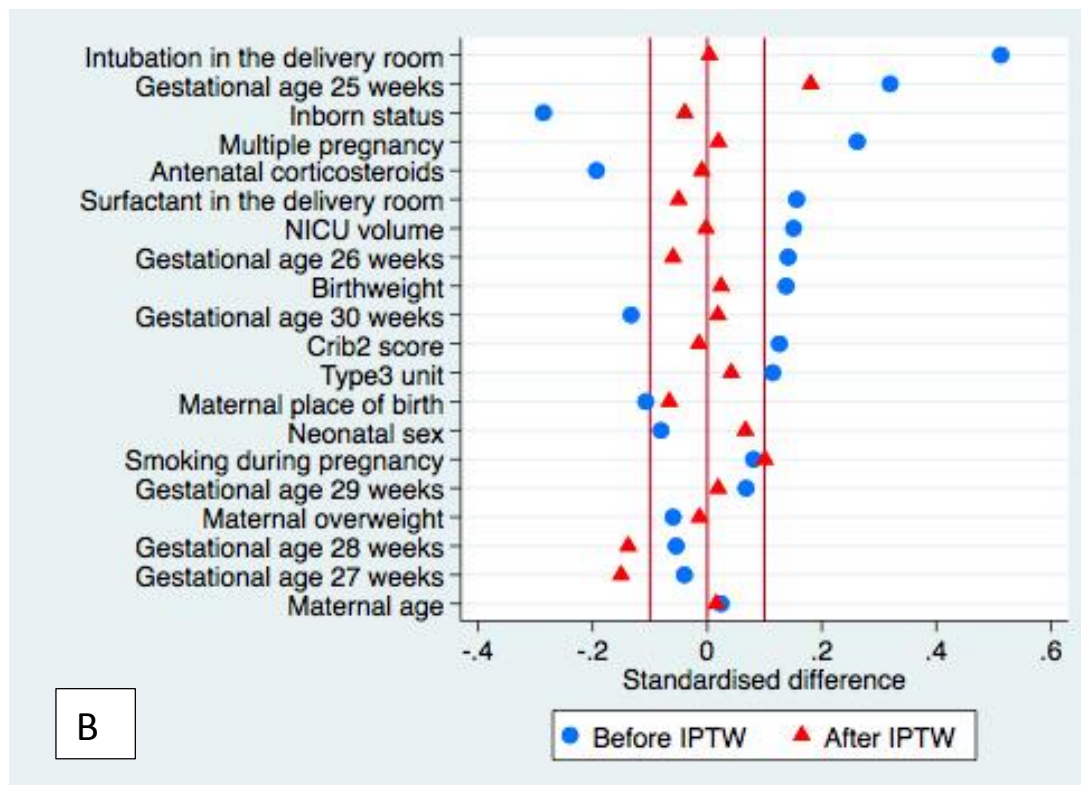
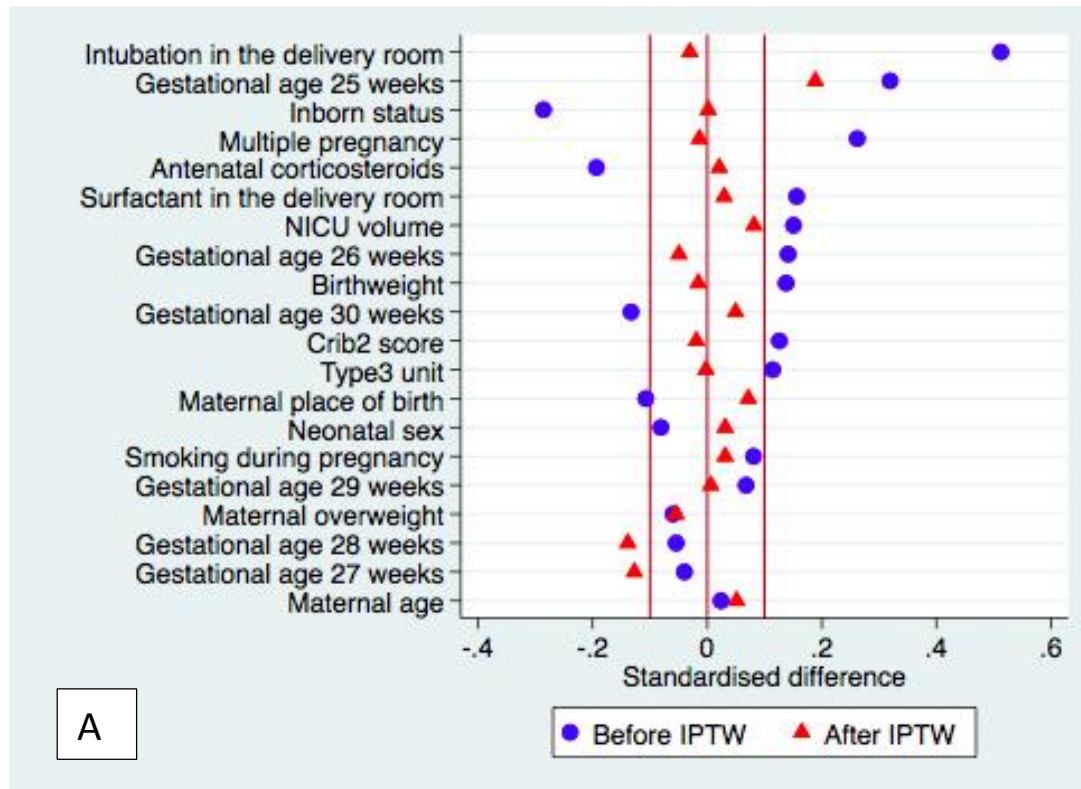
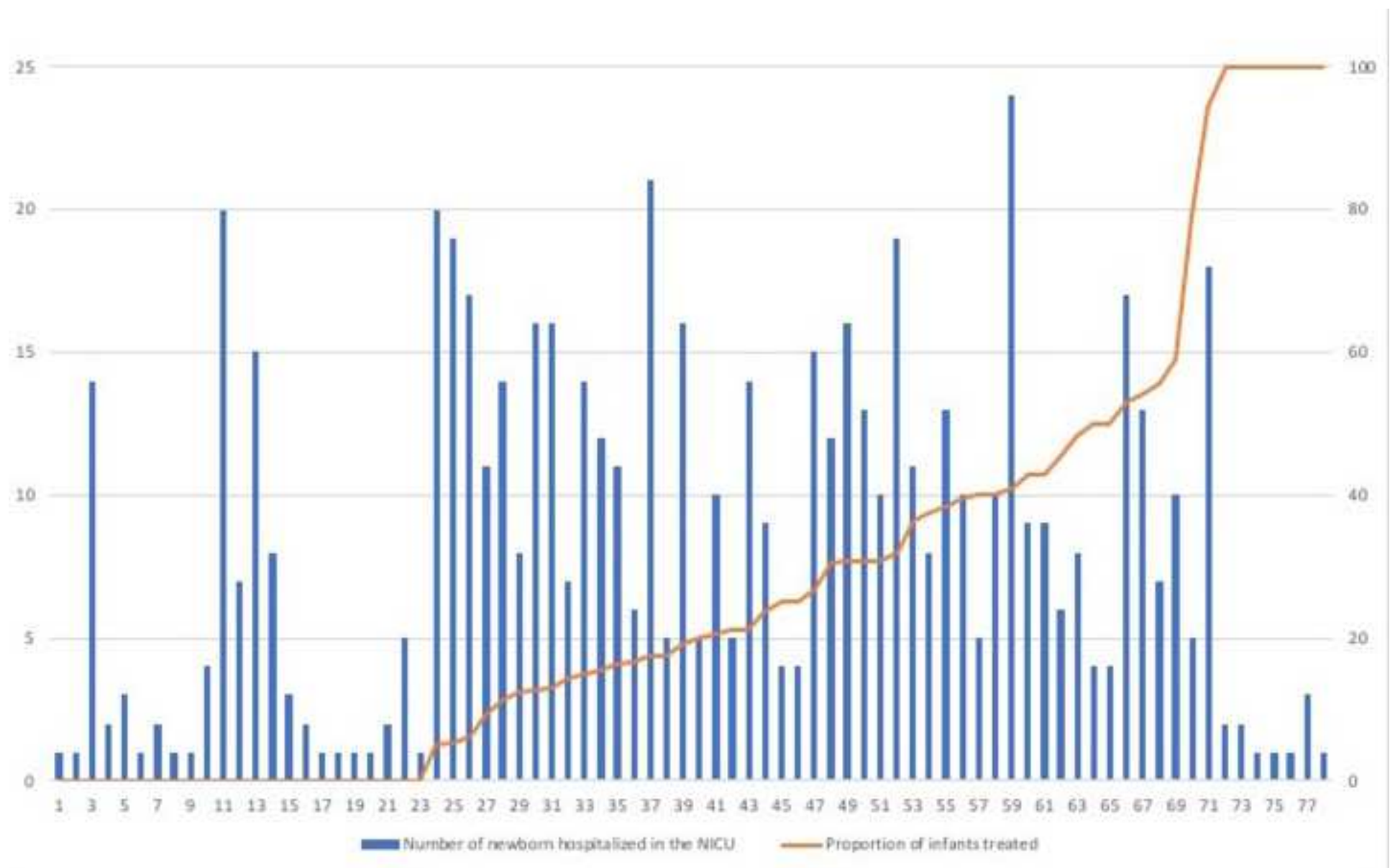


Figure 2 (online): Standardized differences in the initial and IPTW samples in complete-cases (A) and after multiple imputation (B)

Each covariate included in the propensity score is on the y axis.







**Online appendix:** Members of the EPIPAGE-2 Infectious diseases writing group and of the EPIPAGE-2 Study Group

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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	3
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	5-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	8-9
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	9
		(d) If applicable, explain how loss to follow-up was addressed	9
		(e) Describe any sensitivity analyses	9
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Fig1
		(b) Give reasons for non-participation at each stage	Fig1
		(c) Consider use of a flow diagram	Fig1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Tab2
		(b) Indicate number of participants with missing data for each variable of interest	Tab2
		(c) Summarise follow-up time (eg, average and total amount)	Tab2
Outcome data	15*	Report numbers of outcome events or summary measures over time	Tab3

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Tab3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Tab5
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

\*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.