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Article

Elimination of *Candida* Sepsis and Reduction of Several Morbidities in a Tertiary NICU in Greece After Changing Antibiotic, Ventilation, and Nutrition Protocols

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Abstract: Background/Objectives: In recent years, strategies for improving outcomes in preterm neonates have been implemented in various aspects of neonatal care. This study aims to determine the prevalence, microbiology, and outcomes of late-onset sepsis (LOS) and the incidence of other morbidities in very preterm neonates following the implementation of specific infection control, enteral feeding, and ventilation strategies. **Methods:** This study retrospectively compared the morbidity and mortality of neonates with a gestational age <32 weeks over two periods. A series of changes were introduced between these periods, including restrictive use of antibiotics, aggressive enteral feeding, and wider use of non-invasive ventilation modalities. **Results:** A total of 310 neonates were included, 163 in period A (2010-2014), and 147 in period B (2018-2022). The incidence of LOS was 24% and 18%, and of multiple LOS episodes, 26% and 11% in periods A and B, respectively. TPN duration and gestational age were independent predictors of LOS in both periods. The rate of fungal infections declined from 9.2% to 0.7%. Full enteral nutrition in period B was achieved after a median of 7.5 days, compared with 10 days (p=0.001), resulting in fewer days of parenteral nutrition (TPN) (p=0.008). Episodes of feeding intolerance and NEC I significantly reduced (p<0.001). Incidence of intraventricular hemorrhage significantly decreased. **Conclusions:** *Candida* infections were almost completely eliminated. The incidence of LOS and multiple LOS episodes decreased. Early full enteral nutrition was achieved without adverse effects, and fewer episodes of food intolerance were observed. *Candida* elimination appears feasible when antibiotic stewardship is implemented in conjunction with other interventions in a NICU.

Keywords: late-onset sepsis; preterm neonate; neonatal intensive care; antibiotic stewardship; invasive candidiasis

1. Introduction

Preterm birth represents a significant global health concern, affecting approximately 10% of all pregnancies [1]. Prematurity is a major cause of morbidity and mortality in the neonatal population, with an increased risk of short and long-term complications [2]. Among preterm neonates, 15% are born at a gestational age of less than 32 weeks and constitute the most vulnerable population [1].

Improvement of outcomes and reduction of morbidity in preterm neonates represent a considerable challenge, particularly in recent years, given the increase in survival of preterm neonates at younger gestational ages. It is acknowledged that a holistic approach is necessary in the care of the preterm neonate in the neonatal intensive care unit (NICU). In recent decades, neonatologists have focused on implementing new strategies regarding respiratory support, enteral feeding promotion, infection control, and antibiotic reduction in the NICUs to optimize neonatal care and outcomes.

Implementing these practices has improved morbidity and mortality outcomes, but more studies are needed to provide more robust evidence [3-6].

Despite improvements in NICU care, late-onset sepsis (LOS) remains a significant threat to preterm neonates, with prevalence increasing with decreasing gestational age [7,8]. The potentially detrimental consequences in terms of morbidity and mortality, in conjunction with the non-specific presentation and the low sensitivity of diagnostic biomarkers, often lead to the overprescription and administration of unnecessarily prolonged courses of broad-spectrum antibiotics [9-11]. The emergence of multidrug-resistant strains is an inevitable consequence of the excessive use of broad-spectrum antibiotics [9]. Furthermore, an increasing number of studies have demonstrated an association between prolonged antibiotic use in preterm neonates and adverse outcomes, including late-onset sepsis (LOS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), and mortality [12-15]. Therefore, rational and appropriate use of antibiotics in terms of administration criteria, antimicrobial agent choice, and therapy duration is necessary. The implementation of antimicrobial stewardship programs in NICUs aims to promote optimal antibiotic use and minimize short and long-term adverse effects in the neonatal population [16,17].

A significant concern associated with prolonged antibiotic exposure in very low birth weight neonates is the increased risk of invasive candidiasis, particularly in cases when broad-spectrum antimicrobial agents such as third-generation cephalosporins and carbapenems are administered [18,19]. The reduction in broad-spectrum antibiotic use in NICUs has been associated with a decreased incidence of invasive candidiasis, particularly in extremely low birth weight neonates [4,18].

In recent years, following an administrative change, a battery of interventions have been made in this NICU to optimize the outcomes for very preterm neonates by implementing new strategies regarding nutrition, ventilation, and antibiotic administration. We hypothesize that these changes and interventions have led to improved outcomes compared to an earlier period. This study aims to determine the prevalence, microbiology, and outcomes of LOS and the incidence of other morbidities in very preterm neonates following the implementation of certain infection control practices, aggressive enteral feeding, and the wider use of newer ventilation modalities.

2. Materials and Methods

2.1. Study population

The medical records of all preterm neonates with a gestational age of <32 weeks admitted to the NICU of the University Hospital of Ioannina between 2010-2014 and 2018-2022 were retrospectively reviewed for eligibility for inclusion in the study. This is a regional level III NICU covering the entire neonatal population of Northwestern Greece, with 400-450 admissions per year. All very preterm neonates who survived beyond the third postnatal day and had no major congenital anomaly were included in the study. Pregnancy details and neonatal and clinical characteristics from admission to the NICU to hospital discharge were collected for all participants in the study. The study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the Institutional Scientific Review Board at Ioannina University Hospital. Informed consent was obtained from the parents of all neonates involved in the study.

The primary outcome of this study was to determine the prevalence, microbiology, and outcomes of LOS in very preterm neonates following the implementation of certain infection control practices, aggressive enteral feeding, and the wider use of newer ventilation modalities. Secondary outcomes were the incidence of other morbidities such as intraventricular hemorrhage, bronchopulmonary dysplasia, and retinopathy of prematurity.

2.2. Definitions

LOS was defined as a positive blood, urine, and/or cerebrospinal fluid culture beyond 72 hours of age. For *coagulase-negative Staphylococcus* (CoNS) infections to be defined as LOS, two positive cultures and clinical signs of sepsis were required. Pathogens isolated from cultures were categorized into gram-negative bacteria, gram-positive bacteria, CoNS, and fungi. If the same pathogen was isolated in repeated cultures within 7 days, a single episode of sepsis was counted. A central line-associated bloodstream infection (CLABSI) was defined as the isolation of a pathogen from blood culture in neonates with a central line at the time of infection or within the previous 48 hours [20].

The following classification systems were used for the different neonatal morbidities. The severity of IVH was graded according to the Volpe classification [21]. BPD was defined based on respiratory support at 36 weeks postmenstrual age according to the revised National Institute of Child Health and Human Development (NICHD) definition [22]. NEC was classified based on the modified Bell criteria [23]. ROP was classified according to the International Classification of Retinopathy of Prematurity [24]. A neurological examination was conducted on discharge using the Hammersmith Infant Neurological Examination (HINE) [25].

2.3. Differences in practice between the two time periods

In the current study, the morbidity and mortality of preterm neonates <32 weeks of gestational age were retrospectively compared between two time periods. The two periods were 2010-2014 (period A) and 2018-2022 (period B). The decision to compare these two periods was made because, following period A, a series of changes in the care of very preterm neonates were introduced concomitantly in terms of antibiotics administration, nutrition, respiratory support.

In period B, antibiotics with a narrow spectrum and reduced duration were administered. Specifically, in the presence of risk factors for early-onset sepsis (EOS) or suspected LOS, antibiotic treatment was discontinued after 36-48 hours following sterile blood cultures. In period B, the duration of treatment for bloodstream infections was 10 to 14 days. For uncomplicated meningitis, antibiotics were administered for 14 days if gram-positive bacteria were isolated and for 21 days if gram-negative bacteria were isolated. The first-line antibiotics in period B were ampicillin and gentamicin, while teicoplanin, piperacillin/tazobactam, amikacin, or ciprofloxacin were used as second/third line. Third or higher-generation cephalosporins, linezolid, or carbapenems, which were used during period A, were either extremely reduced or eliminated.

With regard to enteral feeding of very preterm neonates in period A, trophic feeding was maintained for five days, followed by a slow advance of 20ml/kg/day. In period B, more aggressive feeding protocols were followed. Trophic feeds (20ml/kg) were maintained for three days in neonates with a birth weight (BW) below 1250g or for one day in neonates with BW>1250g. Following trophic feeding, there was a more rapid increase in milk volume, 30-40ml/kg, depending on birthweight [26].

During period A, mechanical ventilation was more often used for respiratory support for preterm neonates. Conversely, during period B, the systemic utilization of non-invasive ventilation techniques enabled either the avoidance of mechanical ventilation or facilitated early extubation.

3. Statistical analysis

The comparisons between the two study groups of neonates with or without LOS were made respectively either by the t-test or with Mann Whitney U-test after evaluation of parameters for normal or not normal distribution. The Benjamini-Hochberg procedure to adjust for the multiple comparisons was also used. A $p < 0.05$ was considered significant, while values are expressed as mean value \pm standard deviation (SD) or as median and interquartile range (IR). Multiple regression analysis and logistic regression were made to find possible independent parameters related to the presence of LOS. All the parameters that entered the model were tested for collinearity. The Stat View software application of SAS Institute Incorporation (Cary, USA) was used [27].

4. Results

A total of 342 preterm neonates with a gestational age of less than 32 weeks were admitted to the NICU during the two study periods. Of these, 28 were excluded due to early postnatal death (16 in period A, 12 in period B), and 4 were excluded due to major congenital anomalies (1 in period A, 3 in period B). The study included 310 neonates, 163 admitted to the NICU in period A (2010-2014), and 147 in period B (2018-2022).

4.1. Neonatal and obstetrics characteristics

The gestational age and the birth weight of neonates in period A and period B were not significantly different (29.0 ± 2.2 vs. 29.3 ± 2.3 weeks and 1198 ± 325 vs. 1220 ± 367 grams, respectively) (Table 1). There was no difference between the two periods in the proportion of neonates born to multiple pregnancies (44% vs. 42%, $p=0.73$) or through assisted reproductive technologies (32% vs. 35%, $p=0.51$). Antenatal steroid use increased significantly between the two periods, with administration in 70% of pregnancies in period A and 84% in B ($p=0.03$). Vaginal births were less common in period B (14% compared to 25% in period A, $p=0.02$). Maternal chorioamnionitis was diagnosed in significantly more cases in the period B ($p<0.01$).

Table 1. Comparison of obstetric and neonatal characteristics of preterm neonates in the two study periods.

Parameters	Period A (n=163)	Period B (n=147)	p
Sex, male	75 (46%)	83 (57%)	0.06
Gestational age, weeks	29.0 ± 2.2	29.3 ± 2.3	0.35
Birthweight, grams	1198 ± 325	1220 ± 367	0.56
SGA neonates	19 (12%)	25 (17%)	0.17
Multiparity	72 (44%)	62 (42%)	0.73
Maternal age	32.2 ± 5.7	33.7 ± 5.8	0.02
IVF	52 (32%)	52 (35%)	0.51
Antenatal steroids	114 (70%)	124 (84%)	0.003
Maternal chorioamnionitis	2 (2%)	21 (15%)	<0.001
PROM	43 (26%)	43 (29%)	0.57
Mode of delivery, vaginal	40 (25%)	20 (14%)	0.02
Inborn	148 (91%)	135 (92%)	0.74

SGA: small for gestational age; IVF: in vitro fertilization; PROM: premature rupture of membranes.

4.2. Antibiotics

The main changes in antibiotic type and duration between the two periods were the following: the duration of antibiotic treatment in suspected EOS was reduced for a mean of 4 ± 2 to 2 ± 1 days, while in suspected LOS, from 5 ± 2 to 3 ± 1 days, respectively. The mean duration of treatment for LOS was 11.2 ± 4 days compared with 16 ± 4 days in the first period (Table 2). The antibiotics used in the second period were gentamicin and ampicillin as the first line and teicoplanin with piperacillin/tazobactam or ciprofloxacin or amikacin as the second or even third line (in rare occasions). The use of third or higher generations of cephalosporins, linezolid, or carbapenems that were used during the first study period was gradually totally eliminated.

Table 2. Neonatal characteristics of preterm neonates and outcomes in the two study periods.

Parameters	Period A (n=163)	Period B (n=147)	p
Full enteral feeding	10 (6,14)	7.5 (5,10)	0.001
Breast milk	29 (28%)	43 (31%)	0.57
Feeding intolerance	61 (37%)	20 (14%)	<0.001
TPN duration, days	10 (5,22)	7 (5,14)	0.008

Extrauterine growth restriction	88 (61%)	61 (45%)	0.01
Duration of antibiotics in suspected EOS	4±2	2±1	0.001
Duration of antibiotics in suspected LOS	5±2	3±1	0.001
Duration of antibiotics in LOS	16±4	11.2±4	0.001
Central venous catheter	50 (31%)	57 (39%)	0.13
NEC	68 (42%)	29 (20%)	<0.001
NEC III	3 (2%)	2 (1%)	0.72
Ventilation duration, days	3 (0.1,14)	1 (0,7.5)	0.01
Invasive ventilation	129 (79%)	97 (66%)	0.009
BPD	37 (26%)	26 (19%)	0.18
RBC transfusions at 28 days	1 (0,2)	1 (0,2.5)	0.70
ROP	22 (15%)	24 (18%)	0.59
ROP 3	4 (3%)	8 (6%)	0.20
IVH	74 (46%)	38 (27%)	<0.001
IVH III-IV	21 (13%)	9 (6%)	0.04
cPVL	6 (4%)	2 (2%)	0.19
Neurolo			
Neurological discharge, normal	133 (92%)	120 (90%)	0.53
Mortality	18 (11%)	12 (8%)	0.39

TPN: total parenteral nutrition; EOS: early-onset sepsis; LOS: late-onset sepsis; NEC: necrotizing enterocolitis; BPD: bronchopulmonary dysplasia; RBC: red blood cell; ROP: retinopathy of prematurity; IVH: intraventricular hemorrhage; cPVL: cystic periventricular leukomalacia.

4.3. Late-onset infections

In period A, the incidence of LOS was 24% (39 out of 163), and in period B, 18% (27 out of 147). Multiple LOS episodes experienced 26% of neonates (10 of 39) in period A and 11% (3 of 27) in period B (Table 3). The gestational age of neonates with LOS was not significantly different in the two periods ($p=0.52$). The highest incidence of LOS was observed in neonates with a gestational age of less than 28 weeks: 42.3% (22/52) in group A and 33% (14/43) in group B. The rate of CLABSIs was not significantly different between the two periods, with 30% of LOS episodes in period A and 22% in period B ($p=0.39$).

Causative pathogens distribution in period A vs. period B were as follows: CoNS 46% vs. 52% ($p=0.65$), other gram-positive bacteria 13% vs. 12% ($p=0.92$), gram-negative bacteria 33% vs. 42% ($p=0.42$), and fungi 39% vs. 4% ($p<0.001$) (Table 3). The overall rate of fungal infections in the very preterm population was 9.2% in period A and 0.7% in period B. Mortality attributed to LOS was 23% in period A (5 gram-negative, 4 fungal) and 22% in B (6 gram-negative sepsis). In period B, the sepsis-related mortality rate in neonates above 28 weeks was found to be 0% (0/13), in contrast to the 29.4% (5/17) observed in the same gestational age group in period A.

Table 3. Comparison of sepsis prevalence and microbiology in late-onset sepsis in preterm neonates between the two study periods.

Parameters	Period A (n=163)	Period B(n=147)	p
EOS	4 (2.5%)	1 (0.7%)	0.22
LOS	39 (24%)	27 (18%)	0.23
Multiple LOS episodes	10 (26%)	3 (11%)	0.14
Polymicrobial	5 (13%)	1 (4%)	0.13
Gram (+)	5 (13%)	3 (12%)	0.92
CoNS	18 (46%)	14 (52%)	0.65
Gram (-)	13 (33%)	11 (42%)	0.42
Fungi	15 (39%)	1 (4%)	<0.001

CLABSI

10 (30%)

6 (22%)

0.39

EOS: early-onset sepsis; LOS: late-onset sepsis; CoNS: *coagulase-negative Staphylococcus*; CLASBI: central-line associated bloodstream infection.

4.4. Enteral feeding

A significant discrepancy was observed between the two time periods with regard to the time of attainment of full enteral feeding (Table 2). In period A, full enteral nutrition was achieved after a median of 10 days. In contrast, in period B, it was achieved after a median of 7.5 days ($p=0.001$), resulting in fewer days of parenteral nutrition, a median of 10 days in period A and 7 days in period B ($p=0.008$). Despite the more aggressive enteral feeding, episodes of feeding intolerance were significantly lower in period B ($p<0.001$). Episodes of NEC I significantly decreased in period B ($p<0.001$); however, regarding NEC grade II and III, no significant difference was observed.

4.5. Respiratory support

In period A, significantly more neonates received invasive mechanical ventilation (79% vs. 66%, $p=0.009$), and the mean duration of mechanical ventilation was longer (3 vs. 1 day, $p=0.018$). No significant difference was observed in the proportion of neonates that subsequently developed BPD (Table 2).

4.6. Neonatal morbidities and mortality

A significantly lower proportion of neonates developed intraventricular hemorrhage of any grade in period B, 27%, compared to 46% in period A ($p<0.001$) (Table 2). This difference remained significant, although less pronounced, for neonates who developed severe IVH (grade III-IV) (6% in period B vs. 13% in period A, $p=0.49$). The proportion of neonates diagnosed with cystic periventricular leukomalacia (cPVL) remained unchanged ($p=0.19$). Retinopathy of prematurity of any grade and ROP 3 were diagnosed in 15% and 3% of neonates in period A and 18% and 6% in period B, respectively. The overall mortality rate of very preterm neonates, although lower in the second period, did not reach statistical significance, with rates of 11% (18/163) and 8% (12/147) reported in periods A and B, respectively ($p=0.39$) (Table 3).

In a multiple regression analysis at each study period, LOS status (yes or no LOS) was the dependent variable, and the independent variables gestational age, birth weight, duration of mechanical ventilation, duration of antibiotics, number of red blood cell transfusions, age of attainment full enteral feeding, duration of TPN, length of stay in the NICU, presence of BPD and type of feeding. Analysis showed that the only predictors of LOS in both study periods were duration of TPN (OR: 1.12, CI: 1.06, 1.19) and gestational age (OR: 0.84, CI: 0.8, 0.9), while feeding with human milk was also an independent predictor in the second study period (OR: 0.25 CI: 0.1, 0.6).

5. Discussion

The present retrospective comparative study examines the prevalence, microbiology, and outcomes of LOS and the incidence of other neonatal morbidities in very preterm neonates over two periods following changes in preterm care in the NICU regarding antibiotic use, enteral feeding, and ventilation techniques. Regarding LOS, the duration of TPN and gestational age were the only independent predictors.

The prevalence of LOS among very low birth weight neonates was observed to be 24% and 18% in periods A and B, respectively. This rate is comparable to that previously reported by other centers in preterm populations [28-31]. There was a notable reduction, although not statistically significant, in the proportion of newborns with more than one episode of LOS, from 26% to 11%. The vicious circle of antibiotic use in preterm neonates, whereby the administration of antibiotics in the early stages is linked to subsequent infections, has been described previously [13,32]. In the second period of this study, the use of empirical antibiotics was significantly restricted. The decision to administer

antibiotics for EOS was based on risk categorization in accordance with the guidelines published by the American Academy of Pediatrics in 2018 [33]. Furthermore, when antibiotic treatment was initiated based on risk factors or clinical presentation, it was discontinued after 36-48 hours if cultures were negative and the clinical condition was reassuring. The implementation of these strategies resulted in a significant reduction in the duration of empirical antibiotics, from a mean of 4 ± 2 to 2 ± 1 days for suspected EOS and from a mean of 5 ± 2 to 3 ± 1 days for suspected LOS in periods A and B, respectively. In a multicenter study involving 25 NICUs in China, Yu et al. demonstrated that the administration of antibiotics during the first week of life in preterm neonates without culture-proven sepsis resulted in approximately fivefold increased odds of antibiotic use later during the NICU stay. In prolonged or wide-spectrum antibiotic administration, the risk increased by 6–8.5 times compared to neonates that did not receive antibiotics [13].

An important finding of this study was the almost complete elimination of fungal sepsis in the NICU after implementing the strategies described above. Invasive candidiasis is a major cause of concern for the preterm population, with a high rate of morbidity and mortality that is inversely correlated with gestational age and birth weight [34,35]. In a cohort of extremely preterm neonates, the mortality rate was as high as 50%, with almost half of survivors experiencing severe neurodevelopmental impairment [31]. In the first period of the present study, the mortality rate in *Candida* sepsis was 27%. Between the two periods of the study, the rate of fungal infections in the very preterm population declined from 9.2% to 0.7%. Several factors are likely to have contributed to this significant decline, including the restrictive use of antibiotics, the limited duration of parenteral nutrition, and the reduction in the use of invasive ventilation. Notably, the practices regarding antifungal prophylaxis in high-risk neonates remained unchanged throughout the two periods of the study.

In the second period of this study, the strategies implemented regarding the use of antibiotics, with shorter duration and restrictive use of broad-spectrum antibiotics, were expected to reduce the incidence of *Candida* infections. Antibiotics suppress the normal bacterial flora of the gastrointestinal tract, thus diminishing the competitive effect that would naturally inhibit *Candida* proliferation [15]. Aliaga et al., in a large multicenter study, observed a 2.9-7.3% reduction in invasive candidiasis in very low birth weight neonates for every 10% reduction in broad-spectrum antibiotics [4]. The average use of antibiotics per neonate in a NICU, particularly third-generation cephalosporins and carbapenems, has been shown to correlate with the prevalence of systemic *Candida* infections [18,36]. The use of broad-spectrum antibiotics, including third-generation cephalosporins and carbapenems, was largely reduced during the second study period.

In addition to the restrictive use of antibiotics, the reduced duration of parenteral nutrition may have contributed to the elimination of *Candida* infections in the second period of this study. Parenteral nutrition is a recognized risk factor for systemic candidiasis in neonatal populations [37-40]. The prolonged need for vascular access, either by CVC or peripheral venous cannula, results in penetration of epithelial barriers and facilitates *Candida* invasion [35,41]. In addition, the formation of biofilms in implanted medical devices is an important aspect of the pathogenesis of systemic candidiasis. Fungi in these structures are protected from antifungal drugs and the host immune response, and biofilms act as reservoirs for fungal dissemination [42,43]. However, in a previous prospective cohort study, the association between parenteral nutrition and candidiasis was independent of CVC use [39]. Contamination during the preparation of parenteral nutrition and the known effect of lipid emulsions in promoting *Candida* proliferation and the ability to form biofilms are potential contributors to the association between parenteral nutrition and candidiasis [44,45].

Another factor that could potentially contribute to the elimination of *Candida* infections is the reduced use of mechanical ventilation in the second period of the study. In addition to the recognized risk of lung injury and increased risk of chronic lung disease, invasive ventilation disrupts epithelial barriers and predisposes to bacterial and fungal infections [41]. A significant proportion of preterm neonates require respiratory support during the initial postnatal period, which can extend over a range of duration. In recent decades, significant progress has been made in the field of respiratory

support through the widespread use of non-invasive ventilation modalities [46,47]. In the second period of the study, the employment of non-invasive ventilation techniques was broadened. This facilitated either the avoidance of mechanical ventilation or earlier extubation.

In recent years, a growing body of evidence has indicated that the early initiation and more rapid advancement of enteral feeding, even in extremely low birth weight neonates, are beneficial and not associated with increased risk of NEC or other adverse outcomes [48-53]. In the second period of the present study, more aggressive feeding protocols were implemented. These feeding protocols consisted of early initiation and rapid volume advancement, resulting in full enteral feeding achievement at a median of 7.5 days, in contrast to the 10 days in the first period. As anticipated, the attainment of full enteral feeding at an earlier postnatal age resulted in enhanced growth, as evidenced by the significant reduction in the incidence of extrauterine growth restriction. A number of studies have demonstrated a correlation between the administration of parenteral nutrition and its duration with LOS in preterm neonates [54-57]. In the present study, the duration of TPN was identified as the sole independent predictor of LOS in both periods, in addition to gestational age.

In period B, the incidence of suspected NEC (NEC I) decreased significantly compared to period A. This can be at least partly attributed to the restriction of early antibiotic use. The intestinal microbiota of preterm infants has been demonstrated to be more susceptible to disruption by exogenous factors such as early antibiotic exposure [58-60]. A number of studies have evidenced an association between the duration of early antibiotics and NEC [32,61-64]. A recent population-based study of very preterm neonates with no culture-proven sepsis reported that early prolonged empirical antibiotic administration (>5 days) was associated with a more than twofold increase in the odds of developing severe NEC [65]. In the current study, the duration of empirical antibiotics in neonates with suspected EOS and LOS was reduced significantly. However, no decrease in the incidence of surgical NEC was noted.

A significant reduction in intraventricular hemorrhage of any grade was observed in the latter period of the study. The difference in the incidence of IVH between the two periods remained significant, although to a lesser extent when comparing severe IVH. The reduced prevalence of IVH is likely attributable to a combination of factors. In a recent study, Korcek et al., in a large cohort of very preterm neonates, identified the administration of antenatal steroids and the cesarean section as critical factors in the prevention of IVH [66]. In period B of this study, a higher proportion of neonates fulfilled the abovementioned factors compared to period A. In addition, intubation procedure and mechanical ventilation during the first postnatal days have been suggested as potential risk factors for IVH, as they may be associated with fluctuations in cerebral blood flow [67]. However, the observed discrepancy between the two study periods cannot be attributed to a single factor. Instead, it may be attributed to an overall improvement in perinatal care of preterm neonates with a number of measures introduced into everyday clinical practice.

This study has some limitations. The main limitations of the study are its retrospective nature and that it is a single-center study with a relatively modest sample size. Consequently, it is challenging to generalize the results. Furthermore, the impact of other changes in daily practice regarding the care of preterm neonates throughout the study period that were not controlled in the current study cannot be excluded.

6. Conclusions

The almost complete elimination of *Candida* infections, a significant threat to preterm neonates in the NICU, has been the major impact of a series of changes in this NICU. Moreover, a decline in the incidence of neonates with multiple episodes of LOS was observed. The early attainment of full enteral nutrition was achieved without adverse effects, and fewer episodes of feeding intolerance were observed. The results of the current study support the idea of a holistic approach to the care of preterm neonates and further support the application of such changes in the NICU environment. Under these settings, *Candida* eradication appears to be feasible if antibiotic stewardship is implemented in conjunction with other interventions in the NICU.

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References

1. Ohuma, E.O.; Moller, A.B.; Bradley, E.; Chakwera, S.; Hussain-Alkhateeb, L.; Lewin, A.; Okwaraji, Y.B.; Mahanani, W.R.; Johansson, E.W.; Lavin, T.; et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet*. **2023**, *402*, 1261-1271. doi: 10.1016/S0140-6736(23)00878-4. PMID: 37805217.
2. Hall, M.; Valencia, C.M.; Soma-Pillay, P.; Luyt, K.; Jacobsson, B.; Shennan, A.; FIGO Preterm Birth Committee. Effective and simple interventions to improve outcomes for preterm infants worldwide: The FIGO PremPrep-5 initiative. *Int J Gynaecol Obstet*. **2024**, *165*, 929-935. doi: 10.1002/ijgo.15269. PMID: 38264849.
3. Isayama, T.; Iwami, H.; McDonald, S.; Beyene, J. Association of Noninvasive Ventilation Strategies With Mortality and Bronchopulmonary Dysplasia Among Preterm Infants: A Systematic Review and Meta-analysis. *JAMA*. **2016**, *316*, 611-24. doi: 10.1001/jama.2016.10708. PMID: 27532916.
4. Aliaga, S.; Clark, R.H.; Laughon, M.; Walsh, T.J.; Hope, W.W.; Benjamin, D.K.; Kaufman, D.; Arrieta, A.; Benjamin, D.K. Jr.; Smith P.B. Changes in the incidence of candidiasis in neonatal intensive care units. *Pediatrics*. **2014**, *133*, 236-42. doi: 10.1542/peds.2013-0671. PMID: 24446441; PMCID: PMC3904270.
5. Klinger, G.; Bromiker, R.; Zaslavsky-Paltiel, I.; Klinger, S.; Sokolover, N.; Lerner-Geva, L.; Reichman, B; ISRAEL NEONATAL NETWORK. Late-Onset Sepsis in Very Low Birth Weight Infants. *Pediatrics*. **2023**, *152*, e2023062223. doi: 10.1542/peds.2023-062223. PMID: 37786961.
6. Alexander, V.N.; Northrup, V.; Bizzarro, M.J. Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. *J Pediatr*. **2011**, *159*, 392-7. doi: 10.1016/j.jpeds.2011.02.035. PMID: 21489560; PMCID: PMC3137655.
7. Flannery, D.D.; Edwards, E.M.; Coggins, S.A.; Horbar, J.D.; Puopolo, K.M. Late-Onset Sepsis Among Very Preterm Infants. *Pediatrics*. **2022**, Dec 1;150(6):e2022058813. doi: 10.1542/peds.2022-058813. PMID: 36366916; PMCID: PMC11151779.
8. Coggins, S.A.; Glaser, K. Updates in Late-Onset Sepsis: Risk Assessment, Therapy, and Outcomes. *Neoreviews*. **2022**, *23*, 738-755. doi: 10.1542/neo.23-10-e738. PMID: 36316254; PMCID: PMC9675597.
9. Rallis, D.; Giapros, V.; Serbis, A.; Kosmeri, C.; Baltogianni, M. Fighting Antimicrobial Resistance in Neonatal Intensive Care Units: Rational Use of Antibiotics in Neonatal Sepsis. *Antibiotics (Basel)*. **2023**, *12*, 508. doi: 10.3390/antibiotics12030508. PMID: 36978375; PMCID: PMC10044400.
10. Chu, M.; Lin, J.; Wang, M.; Liao, Z.; Cao, C.; Hu, M.; Ding, Y.; Liu, Y.; Yue S. Restrictive Use of Empirical Antibiotics Is Associated with Improved Short Term Outcomes in Very Low Birth Weight Infants: A Single Center, Retrospective Cohort Study from China. *Antibiotics (Basel)*. **2023**, *12*, 741. doi: 10.3390/antibiotics12040741. PMID: 37107103; PMCID: PMC10135071.
11. De Rose, D.U.; Ronchetti, M.P.; Santisi, A.; Bernaschi, P.; Martini, L.; Porzio, O.; Dotta, A.; Auriti, C. Stop in Time: How to Reduce Unnecessary Antibiotics in Newborns with Late-Onset Sepsis in Neonatal Intensive Care. *Trop Med Infect Dis*. **2024**, *9*, 63. doi: 10.3390/tropicalmed9030063. PMID: 38535886; PMCID: PMC10975797.

12. Zini, T.; Miselli, F.; D'Esposito, C.; Fidanza, L.; Cuoghi Costantini, R.; Corso, L.; Mazzotti, S.; Rossi, C.; Spaggiari, E.; Rossi, K.; et al. Sustaining the Continued Effectiveness of an Antimicrobial Stewardship Program in Preterm Infants. *Trop Med Infect Dis.* **2024**, *9*, 59. doi: 10.3390/tropicalmed9030059. PMID: 38535883; PMCID: PMC10975541.
13. Yu, W.; Zhang, L.; Li, S.; Yan, W.; Bai, R.; Yang, Z.; Shi, J.; Yuan, J.; Yang, C.; Cai, W.; et al. Reduction of Infection in Neonatal Intensive Care Units Using the Evidence-based Practice for Improving Quality (REIN-EPIQ) Study Group. Early Antibiotic Use and Neonatal Outcomes Among Preterm Infants Without Infections. *Pediatrics.* **2023**, *151*, e2022059427. doi: 10.1542/peds.2022-059427. PMID: 37042203.
14. Ting, J.Y.; Synnes, A.; Roberts, A.; Deshpandey, A.; Dow, K.; Yoon, E.W.; Lee, K.S.; Dobson, S.; Lee, S.K.; Shah, P.S.; Canadian Neonatal Network Investigators. Association Between Antibiotic Use and Neonatal Mortality and Morbidities in Very Low-Birth-Weight Infants Without Culture-Proven Sepsis or Necrotizing Enterocolitis. *JAMA Pediatr.* **2016**, *170*, 1181-1187. doi: 10.1001/jamapediatrics.2016.2132. PMID: 27775765.
15. Esaïassen, E.; Fjalstad, J.W.; Juvet, L.K.; van den Anker, J.N.; Klingenberg, C. Antibiotic exposure in neonates and early adverse outcomes: A systematic review and meta-analysis. *J. Antimicrob. Chemother.* **2017**, *72*, 1858–1870. doi: 10.1093/jac/dkx088. PMID: 28369594.
16. Dyar, O.J.; Huttner, B.; Schouten, J.; Pulcini, C.; ESGAP (ESCMID Study Group for Antimicrobial stewardship). What is antimicrobial stewardship? *Clin Microbiol Infect.* **2017**, *23*, 793-798. doi: 10.1016/j.cmi.2017.08.026. PMID: 28882725.
17. Notarbartolo, V.; Badiane, B.A.; Insinga, V.; Giuffrè, M. Antimicrobial Stewardship: A Correct Management to Reduce Sepsis in NICU Settings. *Antibiotics (Basel).* **2024**, *13*, 520. doi: 10.3390/antibiotics13060520. PMID: 38927186; PMCID: PMC11200753.
18. Cotten, C.M.; McDonald S, Stoll B, Goldberg RN, Poole K, Benjamin DK Jr; National Institute for Child Health and Human Development Neonatal Research Network. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. *Pediatrics.* **2006**, *118*, 717-22. doi: 10.1542/peds.2005-2677. PMID: 16882828.
19. Hou, S.; Wang, X.; Yu, Y.; Ji, H.; Dong, X.; Li, J.; Li, H.; He, H.; Li, Z.; Yang, Z.; et al. Invasive fungal infection is associated with antibiotic exposure in preterm infants: a multi-centre prospective case-control study. *J Hosp Infect.* **2023**, *134*, 43-49. doi: 10.1016/j.jhin.2023.01.002. PMID: 36646139.
20. Haddadin, Y.; Annamaraju, P.; Regunath, H. Central Line–Associated Blood Stream Infections. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK430891/> (accessed on January 9, 2025)
21. Starr, R.; De Jesus, O.; Shah, S.D.; Borger, J. Periventricular and Intraventricular Hemorrhage. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK538310/> (accessed on January 9, 2025)
22. Higgins, R.D.; Jobe, A.H.; Koso-Thomas, M.; Bancalari, E.; Viscardi, R.M.; Hartert, T.V.; Ryan, R.M.; Kallapur, S.G.; Steinhorn, R.H.; Konduri, G.G.; et al. Bronchopulmonary Dysplasia: Executive Summary of a Workshop. *J Pediatr.* **2018**, *197*, 300-308. doi: 10.1016/j.jpeds.2018.01.043. PMID: 29551318; PMCID: PMC5970962.
23. Patel, R.M.; Ferguson, J.; McElroy, S.J.; Khashu, M.; Caplan, M.S. Defining necrotizing enterocolitis: current difficulties and future opportunities. *Pediatr Res.* **2020**, *88*, 10-15. doi: 10.1038/s41390-020-1074-4. PMID: 32855506; PMCID: PMC8096612.
24. Chiang, M.F.; Quinn, G.E.; Fielder, A.R.; Ostmo, S.R.; Paul Chan, R.V.; Berrocal, A.; Binenbaum, G.; Blair, M.; Peter Campbell, J.; Capone, A. Jr.; et al. International Classification of Retinopathy of Prematurity, Third Edition. *Ophthalmology.* **2021**, *128*, e51-e68. doi: 10.1016/j.ophtha.2021.05.031. PMID: 34247850; PMCID: PMC10979521.
25. Romeo, D.M.; Cowan, F.M.; Haataja, L.; Ricci, D.; Pede, E.; Gallini, F.; Cota, F.; Brogna, C.; Vento, G.; Romeo, M.G.; et al. Hammersmith Infant Neurological Examination for infants born preterm: predicting outcomes other than cerebral palsy. *Dev Med Child Neurol.* **2021**, *63*, 939-946. doi: 10.1111/dmcn.14768. PMID: 33336801.
26. *Guidelines for Acute Care of the Neonate, 26th edition (2018-2019)*; Fernandes, C.J.; Pammi, M.; Katakam, L.; et al. Eds; Baylor College of Medicine, Houston 2014.
27. Altman, D.G. Sample Size. In *Practical Statistics for Medical Research*; Altman, D.G., Ed.; Chapman & Hall: London, UK, 1991; pp. 455–460

28. El Manouni El Hassani, S.; Berkhout, D.J.C.; Niemarkt, H.J.; Mann, S.; de Boode, W.P.; Cossey, V.; Hulzebos, C.V.; van Kaam, A.H.; Kramer, B.W.; van Lingen, R.A.; et al. Risk Factors for Late-Onset Sepsis in Preterm Infants: A Multicenter Case-Control Study. *Neonatology*. **2019**, *116*, 42-51. doi: 10.1159/000497781. PMID: 30947195; PMCID: PMC6690411.
29. Shah, J.; Jefferies, A.L.; Yoon, E.W.; Lee, S.K.; Shah, P.S.; Canadian Neonatal Network. Risk Factors and Outcomes of Late-Onset Bacterial Sepsis in Preterm Neonates Born at <32 Weeks' Gestation. *Am J Perinatol*. **2015**, *32*, 675-82. doi: 10.1055/s-0034-1393936. PMID: 25486288.
30. Boghossian, N.S.; Page, G.P.; Bell, E.F.; Stoll, B.J.; Murray, J.C.; Cotten, C.M.; Shankaran, S.; Walsh, M.C.; Laptook, A.R.; Newman, N.S.; et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. *J Pediatr*. **2013**, *162*, 1120-4, 1124.e1. doi: 10.1016/j.jpeds.2012.11.089. PMID: 23324523; PMCID: PMC3633723.
31. Zhou, Q.; Kelly, E.; Luu, T.M.; Ye, X.Y.; Ting, J.; Shah, P.S.; Lee, S.K. Fungal infection and neurodevelopmental outcomes at 18-30 months in preterm infants. *Front Pediatr*. **2023**, *11*, 1145252. doi: 10.3389/fped.2023.1145252. PMID: 37152326; PMCID: PMC10157087.
32. Cantey, J.B.; Pyle, A.K.; Wozniak, P.S.; Hynan, L.S.; Sánchez, P.J. Early Antibiotic Exposure and Adverse Outcomes in Preterm, Very Low Birth Weight Infants. *J Pediatr*. **2018**, *203*, 62-67. doi: 10.1016/j.jpeds.2018.07.036. PMID: 30172430.
33. Puopolo, K.M.; Benitz, W.E.; Zaoutis, T.E.; COMMITTEE ON FETUS AND NEWBORN; COMMITTEE ON INFECTIOUS DISEASES. Management of Neonates Born at ≤34 6/7 Weeks' Gestation With Suspected or Proven Early-Onset Bacterial Sepsis. *Pediatrics*. **2018**, *142*, e20182896. doi: 10.1542/peds.2018-2896. PMID: 30455344.
34. Ting, J.Y.; Roberts, A.; Synnes, A.; Canning, R.; Bodani, J.; Monterossa, L.; Shah, P.S.; Canadian Neonatal Network Investigators. Invasive Fungal Infections in Neonates in Canada: Epidemiology and Outcomes. *Pediatr. Infect. Dis. J*. **2018**, *37*, 1154-1159. doi: [10.1097/INF.0000000000001968](https://doi.org/10.1097/INF.0000000000001968)
35. Kelly, M.S.; Benjamin, D.K., Jr.; Smith, P.B. The epidemiology and diagnosis of invasive candidiasis among premature infants. *Clin. Perinatol*. **2015**, *42*, 105-117. doi: [10.1016/j.clp.2014.10.008](https://doi.org/10.1016/j.clp.2014.10.008)
36. Chang, Y.J.; Choi, I.R.; Shin, W.S.; Lee, J.H.; Kim, Y.K.; Park, M.S. The control of invasive Candida infection in very low birth weight infants by reduction in the use of 3rd generation cephalosporin. *Korean J Pediatr*. **2013**, *56*, 68-74. doi: 10.3345/kjp.2013.56.2.68. PMID: 23482686; PMCID: PMC3589593.
37. Chen, J.; Jiang, Y.; Wei, B.; Ding, Y.; Xu, S.; Qin, P.; Fu, J. Epidemiology of and risk factors for neonatal candidemia at a tertiary care hospital in western China. *BMC Infect. Dis*. **2016**, *16*, 700. doi: 10.1186/s12879-016-2042-9. PMID: 27884125; PMCID: PMC5121934.
38. Pappas, P.G.; Lionakis, M.S.; Arendrup, M.C.; Ostrosky-Zeichner, L.; Kullberg, B.J. Invasive candidiasis. *Nat. Rev. Dis. Primers*, **2018**, *4*, 18026. doi: [10.1038/nrdp.2018.26](https://doi.org/10.1038/nrdp.2018.26)
39. Saiman, L.; Ludington, E.; Pfaller, M.; Rangel-Frausto, S.; Wiblin, R.T.; Dawson, J.; Blumberg, H.M.; Patterson, J.E.; Rinaldi, M.; Edwards, J.E.; et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. *Pediatr. Infect. Dis. J*. **2000**, *19*, 319-324. doi: 10.1097/00006454-200004000-00011. PMID: 10783022.
40. Menezes, R.P.; Ferreira, I.C.D.S.; Lopes, M.S.M.; de Jesus, T.A.; de Araújo, L.B.; Santos Pedrosa, R.D.; Röder, D.V.D.B. Epidemiological indicators and predictors of lethality associated with fungal infections in a NICU: A historical series. *J. Pediatr*. **2024**, *100*, 267-276. doi: [10.1016/j.jpeds.2023.11.005](https://doi.org/10.1016/j.jpeds.2023.11.005)
41. Fu, J.; Wang, X.; Wei, B.; Jiang, Y.; Chen, J. Risk factors and clinical analysis of candidemia in very-low-birth-weight neonates. *Am. J. Infect. Control*. **2016**, *44*, 1321-1325. doi: [10.1016/j.ajic.2016.03.026](https://doi.org/10.1016/j.ajic.2016.03.026)
42. Junqueira, J.C.; Mylonakis, E. Editorial: *Candida* biofilms. *Front. Microbiol*. **2023**, *13*, 1128600. doi: [10.3389/fmicb.2022.1128600](https://doi.org/10.3389/fmicb.2022.1128600)
43. Wijaya, M.; Halleyantoro, R.; Kalumpiu, J.F. Biofilm: The invisible culprit in catheter-induced candidemia. *AIMS Microbiol*. **2023**, *9*, 467-485. doi: [10.3934/microbiol.2023025](https://doi.org/10.3934/microbiol.2023025)
44. Willems, H.M.E.; Stultz, J.S.; Coltrane, M.E.; Fortwendel, J.P.; Peters, B.M. Disparate *Candida albicans* Biofilm Formation in Clinical Lipid Emulsions Due to Capric Acid-Mediated Inhibition. *Antimicrob. Agents Chemother*. **2019**, *63*, e01394-19. doi: [10.1128/AAC.01394-19](https://doi.org/10.1128/AAC.01394-19)

45. Guducuoglu, H.; Gultepe, B.; Otlu, B.; Bektas, A.; Yildirim, O.; Tuncer, O.; Berktaş, M. Candida albicans outbreak associated with total parenteral nutrition in the neonatal unit. *Indian. J. Med. Microbiol.* **2016**, *34*, 202–207. doi: [10.4103/0255-0857.180303](https://doi.org/10.4103/0255-0857.180303)
46. Harris, C.; Greenough, A. The prevention and management strategies for neonatal chronic lung disease. *Expert Rev Respir Med.* **2023**, *17*, 143-154. doi: 10.1080/17476348.2023.2183842. PMID: 36813477.
47. Kaltsogianni, O.; Dassios, T.; Greenough, A. Neonatal respiratory support strategies-short and long-term respiratory outcomes. *Front Pediatr.* **2023**, *11*, 1212074. doi: 10.3389/fped.2023.1212074. PMID: 37565243; PMCID: PMC10410156.
48. Thoene, M.; Anderson-Berry, A. Early Enteral Feeding in Preterm Infants: A Narrative Review of the Nutritional, Metabolic, and Developmental Benefits. *Nutrients.* **2021**, *13*, 2289. doi: 10.3390/nu13072289. PMID: 34371799; PMCID: PMC8308411.
49. Terefe, A.; Demtse, A.; Abebe, F.; Mislü, E.; Tachbele, E. Predictors of time to full enteral feeding in low birth weight neonates admitted to neonatal intensive care unit: a prospective follow up study. *BMC Pediatr.* **2024**, *24*, 64. doi: 10.1186/s12887-024-04545-0. PMID: 38245699; PMCID: PMC10799381.
50. Young, L.; Oddie, S.J.; McGuire, W. Delayed introduction of progressive enteral feeds to prevent necrotising enterocolitis in very low birth weight infants. *Cochrane Database Syst Rev.* **2022**, *1*, CD001970. doi: 10.1002/14651858.CD001970.pub6. PMID: 35049036; PMCID: PMC8771918.
51. Dorling, J.; Abbott, J.; Berrington, J.; Bosiak, B.; Bowler, U.; Boyle, E.; Embleton, N.; Hewer, O.; Johnson, S.; Juszczak, E.; et al; Controlled Trial of Two Incremental Milk-Feeding Rates in Preterm Infants. *N Engl J Med.* **2019**, *381*, 1434-1443. doi: 10.1056/NEJMoa1816654. PMID: 31597020.
52. Leaf, A.; Dorling, J.; Kempley, S.; McCormick, K.; Mannix, P.; Linsell, L.; Juszczak, E.; Brocklehurst, P.; Abnormal Doppler Enteral Prescription Trial Collaborative Group. Early or delayed enteral feeding for preterm growth-restricted infants: a randomized trial. *Pediatrics.* **2012**, *129*, e1260-8. doi: 10.1542/peds.2011-2379. PMID: 22492770.
53. Morgan, J.; Young, L.; McGuire, W. Delayed introduction of progressive enteral feeds to prevent necrotising enterocolitis in very low birth weight infants. *Cochrane Database Syst Rev.* **2014**, CD001970. doi: 10.1002/14651858.CD001970.pub5. PMID: 25436902; PMCID: PMC7063979.
54. de Sousa, J.C.S.; de Carvalho, A.V.D.; Monte de Prada, L.C.; Marinho, A.P.; de Lima, K.F.; Macedo, S.K.O.; Santos, C.D.P.; da Câmara, S.M.A.; Barreto, A.C.D.N.G.; Pereira, S.A. Nutritional Factors Associated with Late-Onset Sepsis in Very Low Birth Weight Newborns. *Nutrients.* **2021**, *14*, 196. doi: 10.3390/nu14010196. PMID: 35011069; PMCID: PMC8747100.
55. Sohn, A.H.; Garrett, D.O.; Sinkowitz-Cochran, R.L.; Grohskopf, L.A.; Levine, G.L.; Stover, B.H.; Siegel, J.D.; Jarvis, W.R.; Pediatric Prevention Network. Prevalence of nosocomial infections in neonatal intensive care unit patients: Results from the first national point-prevalence survey. *J Pediatr.* **2001**, *139*, 821-7. doi: 10.1067/mpd.2001.119442. PMID: 11743507.
56. Kawagoe, J.Y.; Segre, C.A.; Pereira, C.R.; Cardoso, M.F.; Silva, C.V.; Fukushima, J.T. Risk factors for nosocomial infections in critically ill newborns: a 5-year prospective cohort study. *Am J Infect Control.* **2001**, *29*, 109-14. doi: 10.1067/mic.2001.114162. PMID: 11287879.
57. Kilpatrick, R.; Scarrow, E.; Hornik, C.; Greenberg, R.G. Neonatal invasive candidiasis: Updates on clinical management and prevention. *Lancet Child Adolesc. Health* **2022**, *6*, 60–70. doi: [10.1016/S2352-4642\(21\)00272-8](https://doi.org/10.1016/S2352-4642(21)00272-8)
58. Chernikova, D.A.; Madan, J.C.; Housman, M.L.; Zain-Ul-Abideen, M.; Lundgren, S.N.; Morrison, H.G.; Sogin, M.L.; Williams, S.M.; Moore, J.H.; Karagas, M.R.; et al. The premature infant gut microbiome during the first 6 weeks of life differs based on gestational maturity at birth. *Pediatr Res.* **2018**, *84*, 71-79. doi: 10.1038/s41390-018-0022-z. PMID: 29795209; PMCID: PMC6082716.
59. Arbolea, S.; Sánchez, B.; Milani, C.; Duranti, S.; Solís, G.; Fernández, N.; de los Reyes-Gavilán, C.G.; Ventura, M.; Margolles, A.; Gueimonde, M. Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. *J Pediatr.* **2015**, *166*, 538-44. doi: 10.1016/j.jpeds.2014.09.041. Epub 2014 Oct 25. PMID: 25444008.

60. Xu, Y.; Milburn, O.; Beiersdorfer, T.; Du, L.; Akinbi, H.; Haslam, D.B. Antibiotic exposure prevents acquisition of beneficial metabolic functions in the preterm infant gut microbiome. *Microbiome*. **2022**, *10*, 103. doi: 10.1186/s40168-022-01300-4. PMID: 35794664; PMCID: PMC9260971.
61. Esmaeilizand, R.; Shah, P.S.; Seshia, M.; Yee, W.; Yoon, E.W.; Dow, K.; Canadian Neonatal Network Investigators. Antibiotic exposure and development of necrotizing enterocolitis in very preterm neonates. *Paediatr Child Health*. **2018**, *23*, e56-e61. doi: 10.1093/pch/pxx169. PMID: 30038533; PMCID: PMC6007281.
62. Zhu, K.; Gao, H.; Yuan, L.; Wang, L.; Deng, F. Prolonged antibiotic therapy increased necrotizing enterocolitis in very low birth weight infants without culture-proven sepsis. *Front Pediatr*. **2022**, *10*, 949830. doi: 10.3389/fped.2022.949830. PMID: 36147802; PMCID: PMC9485444.
63. Chen, W.Y.; Lo, Y.C.; Huang, P.H.; Chen, Y.X.; Tsao, P.C.; Lee, Y.S.; Jeng, M.J.; Hung, M.C. Increased antibiotic exposure in early life is associated with adverse outcomes in very low birth weight infants. *J Chin Med Assoc*. **2022**, *85*, 939-943. doi: 10.1097/JCMA.0000000000000749. PMID: 35648148.
64. Cotten, C.M.; Taylor, S.; Stoll, B.; Goldberg, R.N.; Hansen, N.I.; Sánchez, P.J.; Ambalavanan, N.; Benjamin, D.K. Jr.; NICHD Neonatal Research Network. 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. doi: 10.1542/peds.2007-3423. PMID: 19117861; PMCID: PMC2760222.
65. Vatne, A.; Hapnes, N.; Stensvold, H.J.; Dalen, I.; Guthe, H.J.; Støen, R.; Brigtsen, A.K.; Rønnestad, A.E.; Klingenberg, C.; Norwegian Neonatal Network. Early Empirical Antibiotics and Adverse Clinical Outcomes in Infants Born Very Preterm: A Population-Based Cohort. *J Pediatr*. **2023**, *253*, 107-114.e5. doi: 10.1016/j.jpeds.2022.09.029. PMID: 36179887.
66. Korček, P.; Širc, J.; Berka, I.; Kučera, J.; Straňák, Z. Does perinatal management have the potential to reduce the risk of intraventricular hemorrhage in preterm infants? *Front Pediatr*. **2024**, *12*, 1361074. doi: 10.3389/fped.2024.1361074. PMID: 38357510; PMCID: PMC10864433.
67. Gross, M.; Engel, C.; Trotter, A. Evaluating the Effect of a Neonatal Care Bundle for the Prevention of Intraventricular Hemorrhage in Preterm Infants. *Children (Basel)*. **2021**, *8*, 257. doi: 10.3390/children8040257. PMID: 33806111; PMCID: PMC8064449.

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