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Association Between Antibiotic Use and Neonatal Mortality and Morbidities in Very Low-Birth-Weight Infants Without Culture-Proven Sepsis or Necrotizing Enterocolitis

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IMPORTANCE Excessive antibiotic use has been associated with altered bacterial colonization and may result in antibiotic resistance, fungemia, necrotizing enterocolitis (NEC), and mortality. Exploring the association between antibiotic exposure and neonatal outcomes other than infection-related morbidities may provide insight on the importance of rational antibiotic use, especially in the setting of culture-negative neonatal sepsis.

OBJECTIVE To evaluate the trend of antibiotic use among all hospitalized very low-birth-weight (VLBW) infants across Canada and the association between antibiotic use rates (AURs) and mortality and morbidity among neonates without culture-proven sepsis or NEC.

DESIGN, SETTING, AND PARTICIPANTS A retrospective cohort study was conducted among VLBW infants (<1500 g) admitted to level III neonatal intensive care units between January 1, 2010, and December 31, 2014, using data obtained from the Canadian Neonatal Network database.

EXPOSURE Duration of antibiotic use during the hospitalization period.

MAIN OUTCOMES AND MEASURES The AUR was defined as the number of days an infant was exposed to 1 or more antimicrobial agents divided by the total length of hospital stay. The composite primary outcome was defined as mortality or major morbidity, including any of the following: persistent periventricular echogenicity or echolucency on neuroimaging, chronic lung disease, and stage 3 or higher retinopathy of prematurity. Multivariable regression analysis was used to calculate adjusted odds ratios (aORs) and 95% CIs for the association between AURs and outcomes.

RESULTS Among 13 738 eligible VLBW infants, 11 669 (84.9%) (mean [SD] gestational age, 27.7 [2.5] weeks; 47.4% female) received antibiotics during their hospital course and were included in the study. The annual AUR decreased from 0.29 in 2010 to 0.25 in 2014 (slope for the best-fit line, −0.011; 95% CI, −0.016 to −0.006; $P < .01$), which occurred in parallel with a reduction in the rate of late-onset sepsis from 19.0% in 2010 to 13.8% in 2014 during the same period. Of the 11 669 infants who were treated with antibiotics of varying duration during their hospital stay, 2845 were diagnosed as having sepsis-related complications. Among the remaining 8824 infants without early-onset sepsis, late-onset sepsis, or NEC, a 10% increase in the AUR was associated with an increased odds of the primary composite outcome (aOR, 1.18; 95% CI, 1.13-1.23), mortality (aOR, 2.04; 95% CI, 1.87-2.21), and stage 3 or higher retinopathy of prematurity (aOR, 1.18; 95% CI, 1.06-1.32).

CONCLUSIONS AND RELEVANCE Antibiotic use in VLBW infants decreased between 2010 and 2014 in Canada. However, among infants without culture-proven sepsis or without NEC, higher AURs were associated with adverse neonatal outcomes.

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Supplemental content

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Antimicrobial agents are the most commonly prescribed class of medications in neonatal intensive care units (NICUs). In a point prevalence study involving 29 NICUs, Clark et al[1] reported that 47% of infants were receiving at least 1 antibiotic at a particular time. Suspicion of infection is difficult to differentiate from other evolving pathologic processes in preterm neonates because it can progress rapidly, with potentially disastrous consequences.[2,3] Such infection often leads to challenges in the initiation, selection, and duration of antimicrobial therapy.[3] Wide variations in practice among neonatologists regarding the choice and duration of antibiotic treatment for common conditions in the NICU have been reported.[4-6] Inappropriate or excessive antibiotic use has been associated with altered bacterial colonization, resulting in the emergence of resistant organisms and increased rates of fungemia, necrotizing enterocolitis (NEC), and mortality.[7-12] Morbidities, including chronic lung disease (CLD), retinopathy of prematurity (ROP), and periventricular leukomalacia, are associated with systemic inflammatory response, which all may be affected by the intestinal microbial ecology.[13,14] There are a growing number of questions among clinicians, such as when antibiotics should be administered to ensure timely treatment if the index of suspicion is high, when antibiotic use can be justified, and when antibiotic duration can be shortened if the suspicion is low.

The objective of this study was to evaluate the trend of antibiotic use among all hospitalized very low-birth-weight (VLBW) infants across Canadian NICUs. We also examined the association between antibiotic use rates (AURs) and mortality and neonatal morbidities among infants without culture-proven sepsis or NEC.

Key Points

Questions What is the trend of antibiotic use in very low-birth-weight infants across Canada, and is there any association between antibiotic use and adverse outcomes among neonates without culture-proven sepsis or necrotizing enterocolitis?

Findings This cohort study found a significant decrease in antibiotic use among neonatal intensive care unit populations between 2010 and 2014 in Canada. Higher antibiotic use rates were associated with the composite primary outcome of mortality or major morbidity, including persistent echogenicity or echolucency on neuroimaging, stage 3 or higher retinopathy of prematurity, and chronic lung disease.

Meaning Higher antibiotic use rates were associated with adverse neonatal outcomes among infants without culture-proven sepsis or necrotizing enterocolitis.

Methods

Study Population

A retrospective cohort study using data from the Canadian Neonatal Network (CNN) database was conducted, which captures 95% of tertiary-level NICU admissions in Canada. Data were abstracted from infant medical records according to standardized definitions and transmitted to the Canadian Neonatal Network Coordinating Centre in Toronto, Ontario. Data collection and transmission from each site was approved by either research ethics boards or hospital quality improvement committees. Specific institutional review board approval for this study was obtained from the Children's and Women's Research Ethics Board at the University of British Columbia and the Executive Committee of the Canadian Neonatal Network.

Eligibility Criteria

The study included data from VLBW infants (<1500 g) admitted to participating NICUs between January 1, 2010, and December 31, 2014. Infants who had a major congenital anomaly or were missing discharge dates were excluded.

Exposure

The AUR was defined as the number of days an infant was exposed to 1 or more antimicrobial agents divided by the total length of hospital stay. Antimicrobial agents were antibiotics that were prescribed to actively inhibit or kill infecting pathogens according to the CNN drug classification list in the Canadian Neonatal Network *Abstractor's Manual*.[15] Prophylactic administration of trimethoprim or amoxicillin for the prevention of urinary tract infections in patients with a suspected renal anomaly was not included.

Study Variable Definitions

Study variables were defined according to the Canadian Neonatal Network *Abstractor's Manual*. Gestational age (GA) was defined as the best obstetric estimate based on early prenatal ultrasound, obstetric examination, and obstetric history unless the postnatal pediatric estimate of gestation differed from the obstetric estimate by more than 2 weeks. In that case, the pediatric estimate of GA was used instead. An infant was considered small for GA if the birth weight was less than the 10th percentile for GA.[16] The version II Score for Neonatal Acute Physiology (SNAP-II) is a validated measure of newborn severity of illness that captures physiological derangements in the first 12 hours of admission to the NICU.[17] The NICU size was defined as the number of funded (ministry-approved and budgeted) level III beds. For analyses, infants were categorized into 4 groups according to the size of the NICU they were admitted to (<16, 16-29, 30-36, or >36 level III beds). Early-onset sepsis and late-onset sepsis were indicated by positive bacterial, viral, or fungal culture in blood or cerebrospinal fluid from birth to age 2 days and after age 2 days, respectively. Necrotizing enterocolitis was classified as modified Bell stage 2 or higher.[18]

Outcomes

The composite primary outcome was defined as mortality or major morbidity. Major morbidity was defined as the presence of any of the following findings during the hospital stay: persistent periventricular echogenicity or echolucency documented on neuroimaging before discharge,[19] stage 3 through 5 ROP in either eye,[20] and CLD (classified operationally as the receipt of oxygen at 36 weeks' postmenstrual age or at discharge, whichever came first).[21] These 3 outcomes were chosen to be included in this study because of their

association with systemic inflammatory response and their chronological sequence of usually occurring after the median age of infection in preterm neonates. The components of the composite primary outcome were considered secondary outcomes.

Statistical Analysis

The trend of AURs for all patients (with and without infection-related morbidities) during the study period was assessed for significance. To compare patient characteristics of those who did not have sepsis or NEC, patients were divided into approximately 4 equal groups based on their AUR. Comparisons of patient characteristics were performed using descriptive statistics. Adjusted AURs for each year of the study period with 95% CIs were obtained from a regression model. To determine the association between AURs and neonatal outcomes, the adjusted odds ratio for a 10% increase in the AUR and 95% CI were obtained from regression models for infants without sepsis-related complications. For all regression analyses, generalized linear models with appropriate link functions were fitted, adjusting for potential confounders. For the AUR annual trend, the rate was adjusted for GA, sex, SNAP-II exceeding 20, and unit size (4 categories defined by the number of beds at each site). For the association between AURs and neonatal outcomes, the odds ratios were adjusted for GA, sex, SNAP-II exceeding 20, unit size, admission year, small for GA, multiple births, cesarean section, birth at an outside institution, and maternal use of antenatal corticosteroids. A generalized estimating equation with independent correlation structure was used in all regression models to account for correlations among the infants within sites. All statistical analyses were conducted using a software program (SAS, version 9.3; SAS Institute), with statistical significance evaluated using 2-sided *P* values at the 5% testing level.

Results

Overall, there were 13 738 eligible VLBW infants admitted to CNN NICUs between 2010 and 2014. Of these patients, 11 669 (84.9%) (mean [SD] GA, 27.7 [2.5] weeks; 47.4% female) received antibiotics during their hospital course and were included in the study (**Figure 1**).

Trend of Antibiotic Use in VLBW Infants

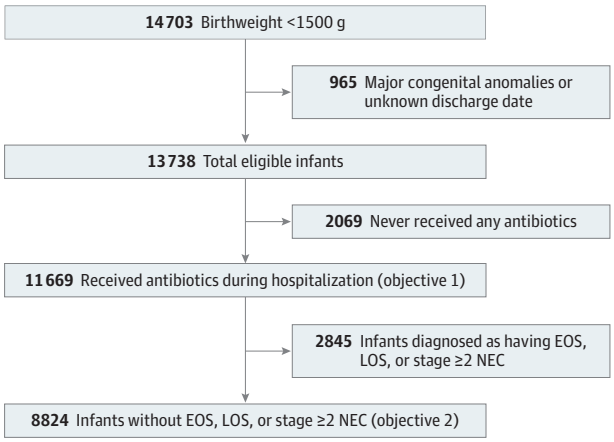
Among eligible VLBW infants, the annual adjusted AUR decreased from 0.29 in 2010 to 0.25 in 2014 (slope for the best-fit line, −0.011; 95% CI, −0.016 to −0.006; $P < .01$), which occurred in parallel with a reduction in the rate of late-onset sepsis from 19.0% in 2010 to 13.8% in 2014 ($P < .01$) (**Table 1**). Among infants without infection-related morbidities during their hospital stay, the adjusted AUR decreased from 0.23 in 2010 to 0.20 in 2014 ($P < .01$) (eTable 1 in the Supplement).

Association Between AURs and Mortality and Major Morbidity Among Neonates

Of the 11 669 infants who were treated with antibiotics of varying duration during their hospital stay, 2845 were diagnosed as having sepsis-related complications (including early-onset sepsis, late-onset sepsis, or stage 2 or higher NEC). The remaining infants (n = 8824) who had no sepsis-related complications were used to evaluate the effect of antibiotic exposure on neonatal outcomes. These infants were divided into 4 groups with approximately equal numbers of patients in each group. The distribution of infant characteristics, maternal factors, and neonatal outcomes is summarized in **Table 2**. Higher AURs were associated with the composite primary outcome of mortality or major morbidity, including persistent periventricular echogenicity or echolucency on neuroimaging, stage 3 or higher ROP, and CLD. Sensitivity analyses for immediate postnatal antibiotic use were conducted by dividing the cohort into 2 groups (AUR≤0.5 vs >0.5) (during the first 7 days of life) and revealed a similar association with all outcomes (eTable 2 in the Supplement). The odds of the composite primary outcome of mortality or major morbidity and individual morbidities were greater among infants with higher AURs within the first 7 days of life (eTable 3 in the Supplement).

Table 3 lists the adjusted odds ratios for a 10% increase in the AUR among infants without infection-related morbidities. Significant increases in the composite primary outcome, mortality, and stage 3 or higher ROP were identified. Treated as continuous variables, the adjusted estimated probability and 95% CI of mortality, the composite primary outcome, and stage 3 or higher ROP are shown in **Figure 2**. Adjusted for 2 continuous variables, GA, and admission year, the figure shows AURs at the mean GA and mean admission year of the sample population used in the study.

Figure 1. Patient Populations in the Present Study



There were 13 738 eligible very low-birth-weight infants admitted to Canadian Neonatal Network neonatal intensive care units between January 1, 2010, and December 31, 2014. Of these infants, 11 669 received antibiotics during hospitalization and were included in the study. EOS indicates early-onset sepsis; LOS, late-onset sepsis; and NEC, necrotizing enterocolitis. Objective 1 was to evaluate the trend of antibiotic use among all hospitalized very low-birth-weight infants across Canada, and objective 2 was to evaluate the association between antibiotic use rates and mortality and morbidity among neonates without culture-proven sepsis or NEC.

Table 1. Infant Characteristics and Antibiotic Use During the Study Period

Variable	2010 (n = 2785)	2011 (n = 2612)	2012 (n = 2762)	2013 (n = 2795)	2014 (n = 2784)
Patient-days, No.	145 963	139 972	146 963	146 611	148 372
Total antibiotic-days, No.	35 156	31 329	30 719	29 593	29 033
Adjusted antibiotic use rate (95% CI)[a]	0.29 (0.27-0.31)	0.29 (0.27-0.31)	0.27 (0.25-0.29)	0.26 (0.24-0.29)	0.25 (0.23-0.27)
Days of antibiotic therapy per patient, median (IQR)	6 (3-16)	7 (3-16)	5 (3-14)	5 (3-14)	5 (3-13)
EOS, No. (%)	24 (0.9)	39 (1.5)	35 (1.3)	38 (1.4)	38 (1.4)
LOS, No. (%)	520 (18.7)	497 (19.0)	430 (15.6)	447 (16.0)	385 (13.8)
Stage ≥2 NEC, No. (%)	166 (6.0)	146 (5.6)	146 (5.3)	187 (6.7)	165 (5.9)
Mortality, No. (%)	258 (9.3)	260 (10.0)	261 (9.4)	267 (9.6)	241 (8.7)

Abbreviations: EOS, early-onset sepsis; IQR, interquartile range; LOS, late-onset sepsis; NEC, necrotizing enterocolitis.

[a] The antibiotic use rate was defined as the number of days an infant was exposed to 1 or more antimicrobial agents divided by the total length of hospital stay. The resulting antibiotic use rate was adjusted for gestational age, sex, version II Score for Neonatal Acute Physiology exceeding 20, and unit size (4 categories defined by the number of beds at each site). The annual decrease for adjusted antibiotic use rate was shown to be statistically significant at $P < .01$ from the model.

Table 2. Infant and Maternal Characteristics and Antibiotic Use Rate in Infants Without Infection-Related Morbidities

Variable	Antibiotic Use Rate Quartile 1 (n = 2193)	2 (n = 2218)	3 (n = 2207)	4 (n = 2206)
Antibiotic use rate, range	0.01	to<0.09 0.09	to<0.17 0.17	to<0.32 0.32 to 1.00
Infant Characteristics				
Gestational age, mean (SD), wk	28.1 (2.0)	28.1 (2.3)	28.1 (2.4)	27.8 (2.9)
Birth weight, mean (SD), g	1101 (235)	1088 (251)	1078 (259)	1047 (308)
Male, No./total No. (%)	1127/2189 (51.5)	1191/2215 (50.5)	1148/2206 (52.0)	1192/2203 (54.1)
Small for gestational age, No./total No. (%)	288/2188 (13.2)	351/2213 (15.9)	352/2206 (16.0)	379/2202 (17.2)
Version II Score for Neonatal Acute Physiology >20, No./total No. (%)	285/2183 (13.1)	375/2212 (17.0)	427/2201 (19.4)	673/2141 (31.4)
Multiple births, No./total No. (%)	758/2193 (34.6)	672/2218 (30.3)	654/2207 (29.6)	643/2206 (29.2)
Cesarean section, No./total No. (%)	1397/2190 (63.8)	1373/2217 (61.9)	1346/2198 (61.2)	1251/2196 (57.0)
Birth at an outside institution, No./total No. (%)	242/2193 (11.0)	310/2218 (14.0)	363/2207 (16.5)	485/2206 (22.0)
Maternal Factors, No./Total No. (%)				
Hypertension	434/2142 (20.3)	441/2172 (20.3)	420/2144 (19.6)	364/2131 (17.1)
Type 1 or type 2 diabetes	226/2109 (10.7)	219/2144 (10.2)	202/2123 (9.5)	198/2115 (9.4)
Use of antenatal corticosteroids	1940/2155 (90.0)	1901/2168 (87.7)	1883/2144 (87.8)	1736/2131 (81.5)
Neonatal Outcomes, No./Total No. (%)				
Composite primary outcome[a]	478/2193 (21.8)	606/2218 (27.3)	659/2207 (29.9)	1013/2206 (45.9)
Mortality	3/2193 (0.1)	14/2218 (0.6)	25/2207 (1.1)	646/2206 (29.3)
Chronic lung disease	398/2187 (18.2)	509/2206 (23.1)	549/2172 (25.3)	321/1559 (20.6)
Persistent echogenicity or echolucency on neuroimaging	65/2141 (3.0)	87/2117 (4.1)	82/2079 (3.9)	111/1706 (6.5)
≥3 Stage retinopathy of prematurity	47/1698 (2.8)	98/1382 (7.1)	131/1166 (11.2)	53/449 (11.8)

[a] The composite primary outcome was mortality or major morbidity, including persistent echogenicity or echolucency on neuroimaging, stage 3 or higher retinopathy of prematurity, and chronic lung disease.

Discussion

To our knowledge, this cohort study is the largest to date to evaluate antibiotic use patterns in NICUs, with a focus on correlations between use pattern and short-term neonatal outcomes in a population-based cohort. We identified reduced nationwide antibiotic use among VLBW infants over time, which corresponded with a decrease in late-onset sepsis rates. We speculate that the concerted effort by all members of the CNN in reducing rates of late-onset sepsis likely resulted in less antibiotic use.[22] Our annual AURs ranged from 0.25 to 0.29 during the study period, which were below the AURs reported in another NICU antibiotic use study[23] comprising 127 NICUs in California (range, 0.26-0.36, depending on the NICU level of care). This finding may be related to regional variations in antibiotic use policies, overall infection rates, or resistance patterns of organisms responsible for late-onset sepsis.

In addition, we discovered that higher AURs during the first 7 days of life in the NICU or the entire hospitalization period were associated with increased neonatal mortality and morbidities. We speculate that the following 2 possibilities

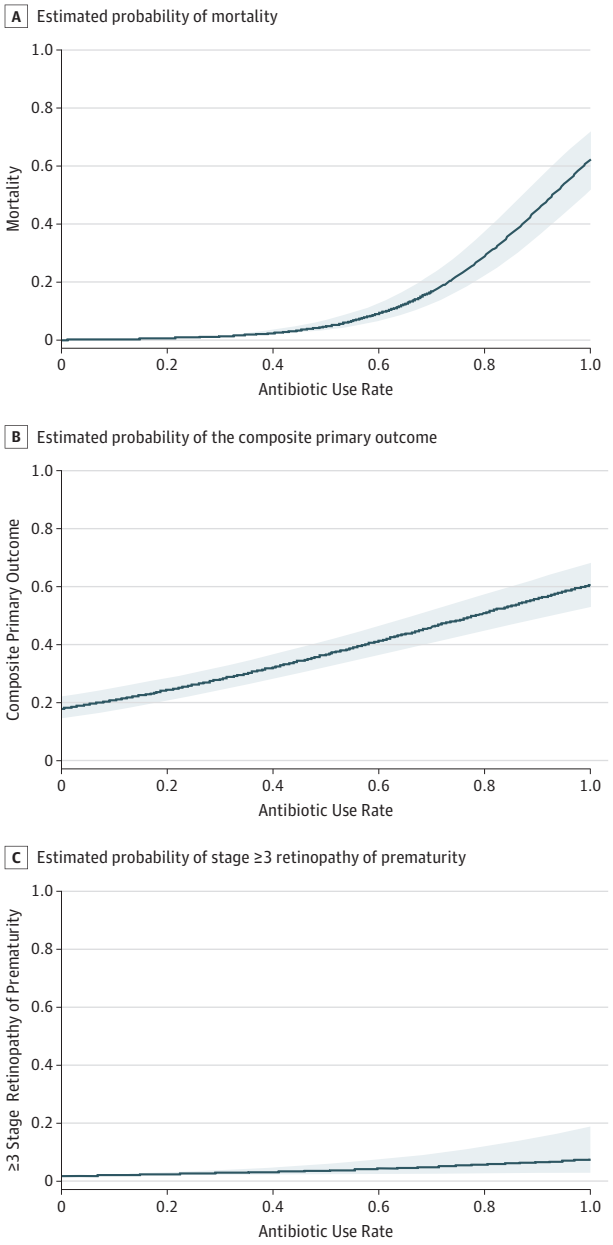
Table 3. Regression Analyses Examining the Neonatal Outcomes in Infants Without Infection-Related Morbidities

Outcome	Adjusted Odds Ratio (95% CI)[a]
Composite primary outcome[b]	1.18 (1.13-1.23)
Mortality	2.04 (1.87-2.21)
Chronic lung disease	1.04 (1.00-1.10)
Persistent echogenicity or echolucency on neuroimaging	1.01 (0.96-1.05)
≥3 Stage retinopathy of prematurity	1.18 (1.06-1.32)

[a] The odds ratio is for a 10.0% increase in the antibiotic use rate adjusted for gestational age, small for gestational age, sex, version II Score for Neonatal Acute Physiology exceeding 20, admission year, multiple births, cesarean section, birth at an outside institution, maternal use of antenatal corticosteroids, and unit size (4 categories defined by the number of beds at each site [<16, 16-29, 30-36, and >36 beds]). A generalized estimating equation with independent correlation structure was used to account for correlations among the infants within sites in the adjusted model.

[b] The composite primary outcome was mortality or major morbidity, including persistent echogenicity or echolucency on neuroimaging, stage 3 or higher retinopathy of prematurity, and chronic lung disease.

Figure 2. Antibiotic Use Rate and Estimated Probabilities of Outcomes



Shown is the estimated probability produced by models adjusted for gestational age and admission year. Fit was computed at the mean gestational age (28 weeks) and mean admission year (2012) of the sample population used in the study. The composite primary outcome was mortality or major morbidity, including persistent echogenicity or echolucency on neuroimaging, stage 3 or higher retinopathy of prematurity, and chronic lung disease.

may explain such an association: (1) the increased exposure to antibiotics may be a risk factor for more morbidities by alterations of microbiota or (2) patients who developed morbidities had a higher rate of systemic inflammatory response and culture-negative sepsis and were more likely to receive antibiotics.

Often, "sick" infants with features indistinguishable from sepsis or early NEC, such as frequent apnea, desaturations, and abdominal distention, or simply those who are "not looking well" have sepsis evaluations performed. The duration of antibiotic use can be a surrogate marker of how sick the infants are. Antibiotics are prescribed for varying durations, depending on culture or other sepsis markers (eg, white blood cell count or C-reactive protein level). While it is understandable to have lower thresholds for empirical antibiotics because of the potentially deleterious outcomes of fulminant sepsis, the duration of therapy for culture-negative sepsis varies widely between and within centers. Moreover, the duration of therapy may not be related to infants' clinical findings or risk index.[24,25] Antibiotics are frequently prescribed in clinical situations in which a clear indication or benefit has not been demonstrated.[26,27] Biomarkers have been extensively studied to differentiate systemic neonatal infection and NEC from other noninfective neonatal conditions that share similar clinical features, but none have entered daily clinical practice.[28] To further complicate the matter, a major dilemma exists when cultures are sterile yet an infant continues to have clinical features or abnormal laboratory results that are consistent with but not necessarily confirmatory of sepsis. Low-colony-count sepsis may not be detected by traditional culture methods because of inadequate volumes of blood collected or because of intrapartum antibiotic prophylaxis.[29-31]

On the other hand, the increased exposure to antibiotics may be a risk factor by itself for more morbidities. Investigations have demonstrated that the increased exposure to broad-spectrum antibiotics, especially third-generation cephalosporins, can increase the risk of neonatal death.[32] By applying high-throughput sequencing (using 16S ribosomal RNA and the beta subunit of RNA polymerase) and quantitative polymerase chain reaction, it has been shown that neonatal antibiotic treatment significantly reduces the amounts of *Bifidobacterium* and *Lactobacillus* in the neonatal gut and impairs intestinal barrier function.[33-35] Perinatal antibiotics, including intrapartum antimicrobial prophylaxis, can also predispose neonates to an increase in Enterobacteriaceae in

the gut mucosa of infants.[36] Infants herein with higher AURs were more likely to have the adverse outcomes of periventricular leukomalacia, ROP, and CLD, even after controlling for GA and severity of illness. Further studies are needed to determine the underlying mechanisms. Given the profound immunoreactivity of the gastrointestinal mucosa, along with the exquisite vulnerability of the neonatal intestinal surface to translocation of inflammatory agents, it is possible that the intestinal microbiota play a critical role in the prevention or pathogenesis of periventricular leukomalacia, ROP, and CLD, all of which have been associated with systemic inflammation.[13,14] In addition, alterations to the infant microbiome secondary to antibiotic use can lead to poor outcomes not only for the individual infant but also for other infants in the NICU through horizontal transmission of pathogens.[37]

Although antibiotics are lifesaving and essential to control bacterial or fungal sepsis, inappropriate or excessive antibiotic use can potentially lead to serious adverse outcomes, including the emergence of multiresistant organisms linked to endemic or epidemic infections, increased rates of invasive candidiasis, NEC, late-onset sepsis, or death.[10-12,38] Prolonged administration of empirical antibiotics to VLBW infants with sterile cultures in the first week of life is associated with subsequent severe outcomes.[10] Researchers have shown that a large proportion of antimicrobial use in NICU settings was not necessary, which prompted the development of antimicrobial stewardship programs similar to those used in pediatric or adult settings.[3,26]

The strengths of our study are that it reflects nationwide Canadian population-based research and evaluation of secular trends of antimicrobial use. Moreover, infants without clear sepsis-related indications were chosen to study the association between antimicrobial agent use and neonatal complications. However, our study has several limitations. First, the neonatal databases did not capture the classes or types of antibiotics that infants received. We were unable to study the causative effect of the use of specific broad-spectrum antibiotics, particularly third-generation cephalosporin or carbapenem groups, and neonatal complications, which have been linked to infectious disease–related adverse events.[9,12,39,40] Second, we may have included some infants with other potential infections (ie, urinary tract infection, pneumonia, or culture-negative sepsis) in analyses of AURs. Standardized definitions of urinary tract infection or pneumonia in preterm neonatal populations were lacking, which made it difficult to estimate the true burden[41,42]; therefore, we included these infants in the study population. Third, we cannot rule out confounding by indication: sicker neonates receive more antibiotics while antibiotic use does not make infants sicker. We attempted to address this limitation by including baseline characteristics and the SNAP-II in the adjustment model; however, the issue of residual confounding remains.

Despite these limitations, the findings of higher AURs associated with neonatal mortality and morbidities are of concern. The results highlight the importance of using antibiotics judiciously in NICU settings, which may minimize the collateral damage associated with antibiotic therapy and benefit neonatal outcomes.[37]

Conclusions

Antibiotic use in hospitalized VLBW infants across Canadian NICUs has decreased over time. Higher AURs were associated with neonatal mortality and morbidities in infants without culture-proven sepsis or NEC. Furthermore, prospective studies that include the duration and type of antibiotics used may identify opportunities for practice improvement that can reduce the development of adverse neonatal outcomes.

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