ANTIBIOTIC PRESCRIBING DURING INFANCY AND RISK OF TREATED OBSTRUCTIVE AIRWAY DISEASES DURING EARLY CHILDHOOD: A REGISTRY-BASED NATIONWIDE COHORT STUDY IN DENMARK

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ABSTRACT

Alan C. Kinlaw: Antibiotic Prescribing during Infancy and Risk of Treated Obstructive Airway Diseases during Early Childhood: A Registry-Based Nationwide Cohort Study in Denmark
(Under the direction of Til Stürmer)

Widespread antibiotic use leads to bacterial resistance, and antibiotic use in early life may be associated with asthma in childhood. To date, studies of this association have led to inconsistent findings. Additionally, data are limited regarding cohort effects on antibiotic use in children, which may impact underlying susceptibility to adverse effects.

Using nationwide registry data on all children born in Denmark during 2004-2012, our objectives were to (1) examine birth-season and birth-year cohort effects on antibiotic prescribing during the first year of life (henceforth, 'infancy'), and (2) to estimate 1-, 2-, and 3-year risk differences (RD) for the association between antibiotic prescribing during infancy and treated airway diseases from 2-5 years of age, using propensity scores (PS) and instrumental variables.

The 1-year risk of redeeming at least one antibiotic prescription during infancy was 39.5 per 100 children. The hazard of first redeemed antibiotic prescription increased with age throughout infancy, and peaked in February; as a result, season of birth impacted overall 1-year risk of redeeming an antibiotic prescription during infancy and age at first redeemed antibiotic prescription. Amoxicillin prescribing was dynamic over the study period, but decreased after

distribution of a bulletin on rational antibiotic use in general practice and rollout of two nationwide pneumococcal vaccination programs.

In PS analyses, antibiotic exposure was associated with increased risk of treated airway diseases by age 5, compared with no exposure (3-year RD = 4.5 per 100 children, 99% confidence interval (CI): 4.2, 4.8). PS-based dose-response analysis suggested that each additional redeemed antibiotic prescription was associated with increased risk of 2.4 per 100 children (99% CI: 2.3, 2.5). RDs were negligible in a PS-based head-to-head comparison between two antibiotics with similar indications but differing spectrum of antibacterial activity – amoxicillin and penicillin V (3-year RD = -0.1 per 100 children, 99% CI: -0.6, 0.3). Results from instrumental variable analyses also cast some doubt on the presence of a causal effect, but were imprecise. These results suggest that antibiotic exposure during infancy may increase the risk of treated airway diseases, but further exploration is needed using data and methods capable of addressing potential residual confounding.

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LIST OF ABBREVIATIONS

aOR	adjusted odds ratio
ATC	Anatomic Therapeutic Chemical
ATE	average treatment effect in the population
ATT	average treatment effect in the treated
BMI	body mass index
CB	confidence band
CI	confidence interval
CPR-number	Personal registration number
CRS	Civil Registration System
DTaP/IPV/Hib	Diphtheria/Tetanus/acellular Pertussis/Polio/Haemophilus influenzae type b
GP	general practitioner
HR	hazard ratio
ICD	International Classification of Diseases
IgE	immunoglobulin E
IPT	inverse-probability-of-treatment
IQR	interquartile range
IRF	Institute for Rational Pharmacotherapy
ITT	intention-to-treat
IV	instrumental variables
kg	kilogram
km	kilometer
LATE	local average treatment effect

LRTI	lower respiratory tract infection
m	meter
MBR	Medical Birth Registry
mw	mean weight
NHSPD	National Health Service Prescription Register
NICU	neonatal intensive-care unit
NNT	number needed to treat
NNTH	number needed to treat to harm
NPR	National Patient Registry
OR	odds ratio
PCV13	13-valent pneumococcal conjugate vaccine
PCV7	7-valent pneumococcal conjugate vaccine
PS	propensity scores
RD	risk difference
RR	risk ratio
RSV	respiratory syncytial virus
RV	rhinovirus
SAMD	standardized absolute mean difference
SMD	standardized mean difference
SMR	standardized morbidity ratio
Th1	T-helper type 1
Th2	T-helper type 2
URTI	upper respiratory tract infection

CHAPTER 1: STATEMENT OF SPECIFIC AIMS

Asthma is one of the most common chronic diseases in childhood, and poses a significant worldwide burden.[1–3] Childhood asthma prevalence ranges 3-7% in Denmark,[4,5] and approximately 10% of children take prescription medication to treat related symptoms.[6,7] The prevalence of asthma, atopic response, and other allergic conditions has increased in recent decades,[1,2,8–12] especially in industrialized areas,[3,13,14] but reasons for these increases are unclear.[1,2,15–19]

Many causes have been hypothesized to explain observed increases in asthma prevalence.[13,14] Along with genetics,[20,21] environmental factors,[20–24] and viral infection,[20,21,24] the "hygiene hypothesis"[25] may also explain recent increases in the prevalence of childhood asthma, especially in industrialized countries.[14] This controversial[19] hypothesis asserts that adequate microbial exposure is important for developing proper immune response in early life.[19,26] Subsequently, child development in an overly hygienic environment – with lower exposure to microbiota – may induce elevated atopic response in children and elevated risk of the development of asthma.[7,14,27–29]

Given that antibiotics deplete and disrupt bacterial flora in the gut,[30,31] they have long been suspected to cause increased risk of atopic immune response and asthma, particularly in children.[14,32] Over the last two decades, numerous epidemiologic studies have examined the potential association between childhood antibiotic exposure and asthma,[12,14,26,33–37] yielding

conflicting results.[13,38] Each prior study has been limited by intractable biases, including confounding by indication or other unmeasured factors, reverse causality, other protopathic bias, and recall bias.

Antibiotic prescribing is common in children,[32,39] and unnecessary use frequently occurs because bacterial and viral variants of upper respiratory tract infections are often clinically indistinguishable.[40–43] Decreasing unnecessary antibiotic prescribing is a critical feature of clinical and public health approaches to stifle mounting threats of population-level bacterial resistance.[32,40,44–47] In western industrialized countries in particular, increasing advocacy for rational antibiotic prescribing has been associated with decreases in population-level antibiotic use.[48–51] In addition, pneumococcal conjugate vaccination programs have been associated with decreased risk of acute otitis media and lower respiratory tract infections,[52] two of the most common indications for antibiotics in children.

The overall goals of this research are to characterize the changing patterns of antibiotic prescribing in children to inform future studies of antibiotic effectiveness and safety, and to examine the relation between early life antibiotic prescribing and the development of obstructive airway diseases in childhood. This dissertation has two primary aims:

1.1. SPECIFIC AIM 1

Describe antibiotic prescribing patterns during the first year of life among children born in Denmark during 2004-2012, with attention to birth-month, birth-season, and birth-year cohort effects. Additionally, part of this investigation addresses the potential impact of two population-level changes during this time period:

(1) a nationwide bulletin issued from the Danish Health and Medicine Authority's Institute for Rational Pharmacotherapy (IRF) to general practitioners in April 2007 with guidelines for rational antibiotic prescribing,[47] and

(2) the Danish childhood vaccination program's rollout of the 7-valent pneumococcal conjugate vaccine (PCV7) in October 2007 and the 13-valent vaccine (PCV13) in April 2010.[53–55]

1.2. SPECIFIC AIM 2

Assess the association between antibiotic prescribing during the first year of life and incidence of treated obstructive airway diseases during early childhood, among children born in Denmark during 2004-2012. Based in part on evidence from Aim 1, this investigation focuses on estimating risk differences for the effect using propensity scores[56,57] and instrumental variables[58–60] to address bias, including assessment of a dose-response relation and heterogeneity of effects by age at first exposure to antibiotics. The individual-level analysis in sub-aim 2a reduces potential confounding to estimate associations between antibiotic prescribing and treated airway diseases in childhood; the ecologic analysis in sub-aim 2b uses instrumental variables under the potential outcomes methodological framework[58] intended to assess explicit causal associations between antibiotic prescribing and treated airway diseases.

Sub-aim 2a

Conduct an individual-level analysis of the relation between exposure to antibiotics during the first year of life and incident treated airway diseases in early childhood. Using linked registry data with rich covariate information on family medical history, pregnancy, infant health,

hospital and clinic visits, prescriptions and demographics, this assessment relies on propensity score-based[56,61–63] weighting methods[64,65] to reduce confounding and to estimate population-level measures of absolute risk of treated airway diseases across levels of antibiotic exposure, including head-to-head comparisons between amoxicillin and penicillin V, dose-response analysis, and an assessment of risk difference heterogeneity by age at first exposure.

Sub-aim 2b

Conduct time-based ecologic analyses related to population-level occurrence of antibiotic prescribing and treated airway disease. Using birth-season cohort and calendar time as instrumental variables,[58–60,66,67] this analysis estimates effect estimates analogous to a randomized controlled trial with non-compliance. Instrumental variable analysis is conditional on three assumptions, which in the context of this study are as follows: (1) the instrument affects the proportion of children exposed to antibiotics; (2) the instrument is unrelated to other covariates such that the association between the instrument and risk of treated airway diseases is not confounded; and (3) the instrument does not directly affect the risk of treated airway diseases. Together, these assumptions imply that the instrument can only be associated with the risk of treated airway diseases if antibiotic exposure has an effect on treated airway diseases.[58,60,68]

CHAPTER 2: REVIEW OF THE LITERATURE

2.1. BACKGROUND

Asthma is a major chronic disease worldwide and its prevalence is increasing.

Asthma is one of the most common chronic conditions among children worldwide.[1,3,20,69,70] Asthma-related mortality among children is relatively low compared with other health conditions,[71] but it impacts overall health and quality of life.[1] Especially when compared to other illnesses that may be non-fatal,[72] asthma imposes one of the heaviest burdens of disease in the world today.[1,70,72,73] Prevalence of childhood asthma ranges from 2-11% across world regions,[3,74,75] and from 3-7% in Denmark.[4,5] In Denmark, risk of asthma has been estimated at 10%[76] and 6%[77] by 7 years of age; 14%[78] and 5%[79] by 10 years of age; and 5%[80] by 12 years of age.

The prevalence of asthma, atopic response, and other allergic conditions has increased in recent decades,[1,2,8–12,14] especially in industrialized areas,[3,13,14] and asthma is now the most common chronic condition among children worldwide.[69] Based on preliminary data,[81] approximately 5-10% of children in Denmark aged 5-14 years were prescribed medication for treatment of obstructive airway diseases between 2000 and 2013. These observations are similar to those in the United States, where approximately 10% of children and 5% of adults were prescribed medication for treatment of asthma in 2006.[3,7,14,82]

Despite recognition of population-level increases in asthma, reasons for these increases are unclear.[1,2,15–19] Many causes have been hypothesized to explain observed increases in asthma prevalence;[13,14] however, as the underlying causal framework of asthma morbidity remains unestablished,[20,21] so does our understanding of changes in its prevalence. Asthma is an inheritable condition, but relationships between genetic factors and asthma phenotypes are not clearly defined and are not sufficient to explain increases in occurrence of asthma.[20,21] Increasing childhood asthma prevalence may also be caused by changing environmental factors such as increased airborne allergen levels (e.g., house dust mites and *Alternaria* fungi);[20,22,23] respiratory infection by rhinovirus (RV) and respiratory syncytial virus (RSV);[20,21] and increased urbanization, air pollution, chemical irritants, and tobacco smoke.[7,13,14,20,21,26,69]

The "hygiene hypothesis" is a biologically plausible explanation of increasing asthma.

In addition to those potential causes mentioned above, the "hygiene hypothesis"[25] may also explain recent increases in the prevalence of childhood asthma, especially in industrialized countries.[14] This controversial[19] hypothesis asserts that adequate microbial exposure is important for developing proper immune response in early life.[19,26] This hypothesis originated from observations of individuals with high exposure to microbiota with less incident respiratory problems than those with lower exposure levels,[25] and has been extended to offer an explanation for observed increases in asthma and atopy among individuals with low exposure to microbiota. Subsequently, child development in an overly hygienic environment – with lower exposure to microbiota – may induce elevated atopic response in children and elevated risk of the development of asthma.[7,14,27–29]

In infants and adults, bacterial flora in the gut is impacted by host genetics and can be depleted by infection, chemotherapy and radiation; depletion is also thought to occur following exposure to antibiotics and might explain the onset of suboptimal immune function following anti-infective treatment.[30,83] Factors in early life which are suspected to increase the child's microbial exposure include vaginal delivery (versus cesarean), later gestational age at birth, breastfeeding during infancy, as well as bacterial characteristics, human mucosal cell characteristics, and child antibiotic use.[13,24,84–86] Experiments in mice have also demonstrated that microbial exposure *in utero* protects against allergic phenotypes and that sufficient bacterial gut colonization in early life plays an important role in immune response programming.[14,87–90] Observational studies in children have also suggested that prenatal antibiotic exposure may also increase risk of asthma;[91–94] however, it is important to note that potential associations between antibiotic exposure and asthma in the prenatal and postnatal period are likely based on distinct biologic mechanisms, given that the human fetal gut is thought to remain sterile *in utero*.[95]

Proper immune response is generally characterized as a balance between T-helper type 1 (Th1) and T-helper type 2 (Th2)[96,97] cytokine responses.[20] Th1 cytokine (e.g., interferon-γ) responses are largely for proinflammatory killing of intracellular organisms, whereas Th2 responses promote immunoglobulin E (IgE) antibodies to combat multicellular helminths, and are more anti-inflammatory.[19,21,97] A Th1-skewed response profile could result in tissue damage, and a Th2 skew could result in asthma and other atopic phenotypes.[97] These potential abnormalities underscore the need for adequate balance of the Th1/Th2 mechanisms for proper immune response.[20,21,97]

The natural history of the early life Th1/Th2 balance is essential to the proposed study of antibiotic exposure during infancy and childhood asthma, because this balance impacts infant

susceptibility and response to infection. During pregnancy, the balance is Th2-skewed because the fetal environment is largely sterile[95] and autoimmune response could lead to preeclampsia or spontaneous abortion.[21] After experiencing its first major contact with microflora by traveling through the birth canal, the healthy infant's naïve, Th2-skewed and hyporesponsive immune system adapts slowly over the course of childhood to a more balanced and robust state.[21,98] However, insults to the naïve infant immune system – by such means as repeated viral infection or antibiotic exposure – may lead to immune dysfunction in later childhood years.[98]

Antibiotic prescribing to children often occurs for viral infections and may often be unnecessary.

Out of all antibiotic prescriptions to children, nearly one-third occur for non-bacterial upper respiratory tract infections[14,26,28,29,32,99] for which they are not effective.[32,100] Although there is some interest in macrolide antibiotics – Anatomic Therapeutic Chemical (ATC) group J01FA[101] – as potentially beneficial in treatment of infection due to their anti-inflammatory properties, there is otherwise very little evidence for benefit to children prescribed antibiotics for viral infection.[21,100]

Most acute upper respiratory tract infections (e.g., bronchitis, sinusitis, pharyngitis) are viral and self-limiting.[32,40,102] In a small proportion of children, these viral infections can be accompanied or followed by secondary bacterial infection(s), including acute otitis media, sinusitis, and pharyngitis.[32,40,102] The common pathogens that cause acute otitis media and sinusitis are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*,[45,103] whereas *Streptococcus pyogenes* and *Mycoplasma pneumoniae* are the primary bacterial causes of pharyngitis.[104] Antibiotics are prescribed to children for treatment of upper respiratory tract infections because (a) bacterial and viral infections are often clinically indistinguishable among

children with variable levels of atopic response,[40,41] and (b) antibiotics are protective against secondary suppurative complications from bacterial infection.[32,40,102] In settings with low access to healthcare, prophylactic antibiotic prescribing may be more sensible than in settings with high access to healthcare, where watchful waiting[45] can be used and children who show symptoms of bacterial infection at a proximate follow-up visit can subsequently be prescribed an antibiotic.

These complications (e.g., mastoiditis, pneumonia, peritonsillar abscess) are serious outcomes, but rare among children with upper respiratory tract infection, on the order of <0.1%.[32 32,45,105,106] For example, although antibiotics are effective for preventing mastoiditis, the incidence of mastoiditis is extremely low regardless of antibiotic treatment; in order to prevent one case of mastoiditis among children, two studies have estimated that >4,800[106] and >12,000[107] cases of otitis media would require antibiotic treatment. Such a high magnitude for the number needed to treat (NNT) estimand may elucidate some of the complexity surrounding opinions on antibiotic prescription recommendations.

Antibiotic exposure in infancy and early childhood is modifiable.

In the United States pediatric population in 1992, approximately 40% of doctor visits for viral infections resulted in an antibiotic prescription.[99] In Europe, providers' misconceptions of the risks and benefits pertaining to antibiotic prescription has been associated with increased prescribing.[42] With such data in mind to inform both practitioners and parents, it is feasible that antibiotic prescriptions can be reduced at the population level and targeted more appropriately. As information has increasingly been disseminated over the last two decades about antibiotic effectiveness and bacterial resistance to subsequent antibiotic treatments,[32,40,44] a burgeoning culture of increasingly judicious prescribing[51] has resulted in decreases in antibiotic prescription,

especially in children.[48–50] The continuing reduction of unnecessary and excessive prescription of antibiotics to infants and children is possible. Such a modification would curb the frequency and extent of microbial resistance to antibiotics,[32,40,48] reduce incidence of other unintended adverse effects of antibiotics,[32,85] and decrease unnecessary financial expenditures for antibiotics and further acute clinical consultation.[32]

2.2. SOURCES OF BIAS IN STUDIES OF EARLY LIFE ANTIBIOTIC EXPOSURE AND CHILDHOOD ASTHMA

The potential etiologic relation between infant antibiotic exposure and childhood asthma is difficult to assess due to uncertainty about the biologic mechanism and potential bias in epidemiologic studies. Asthma pathogenesis is highly complex,[20] and investigators conducting observational studies have (justifiably) been criticized for narrowing their scope of inquiry toward straightforward explanations of potential risk factors for asthma.[19,34] With respect to the 'hygiene hypothesis,' in studies of antibiotic exposure and asthma, interpretation of results is usually carried out with implications toward acceptance or rejection of the 'hypothesis,' couched in language suggestive of causal structures, despite (always present and sometimes severe) limitations to inference. Furthermore, there are several biases that have likely impacted the results published so far on early antibiotic exposure and asthma. Each of these is described with a relevant example below.

Protopathic bias

Protopathic bias,[7,14] a type of reverse causality bias, can arise when underlying preclinical symptoms of the outcome of interest affect the treatment of interest. In a study of antibiotics and asthma, this could manifest if infants showing symptoms of underlying asthma are more likely to be treated with antibiotics prior to the asthma diagnosis being made. This would tend to bias results upward.

(Other) reverse causality bias

Other reverse causality bias can occur more generally when temporality of exposure (the hypothesized causal variable) and outcome is ambiguous. This could happen in a study of antibiotic exposure and asthma if (a) exposure and outcome ascertainment took place at or near the same time (e.g., in a cross-sectional study), or (b) if outcome ascertainment were for prevalent asthma or history of asthma.[14]

Confounding bias by indication

Confounding by indication[108] can arise when the indication for treatment is an independent risk factor for the outcome. It can occur if infants are prescribed antibiotic treatment for an illness (e.g., infection with RV or RSV[20,21]) which is an independent risk factor for asthma. In such a scenario, risk factors for asthma would be an indication for antibiotic prescribing. In an analysis of this scenario, the association between antibiotic prescribing and asthma incidence would be confounded by the risk factor, leading to upward bias.

(Other) confounding bias

Confounding bias[109] can arise when an independent risk factor for the outcome varies across levels of exposure and is not a causal intermediate between exposure and outcome. There are many ways in which confounding bias could occur in a study of antibiotics and asthma. For example, individuals in large cities receive more antibiotic prescriptions than those in rural areas; likewise, living in urban environments increases risk of asthma independently of antibiotic prescribing. A crude analysis of a random subset of the source population, comparing exposed versus unexposed regardless of home location, would therefore be confounded by home location and the magnitude of this confounding would be, in part, dependent on the proportion of the source population living in urban environments.

Selection bias

Selection bias[110] can arise when the relation between exposure and outcome in a study population differs from the relation between exposure and outcome in the target population. Assuming no other bias, if selection is associated with exposure and outcome, then absolute and relative estimates will be biased; if selection is associated with exposure only, then relative estimates will be valid; if selection is independent of exposure and outcome, then absolute and relative estimates will be valid. Selection bias could occur in a study of antibiotics and asthma if, for example, the study population contained a higher proportion of infants with parental history of asthma than the target population. In this setting, their underlying characteristics and risk for asthma would probably be different from those in the overall target population.

Recall bias

Recall bias[111] arises in cross-sectional or retrospective studies when reporting of exposure occurs differentially with respect to the outcome or other characteristics. This could arise in a cross-sectional or case-control study of antibiotics and asthma in which a parent of an asthmatic child might be more likely to report early antibiotic exposure than a parent of a non-asthmatic child,[108] leading to upward bias. Likewise, for children with and without asthma, poor recall of antibiotic exposure (e.g., among parents with older or multiple children) could result in non-differential misclassification bias toward the null.

Immortal time bias

Immortal time bias[112,113] can arise when exposure categories are assigned follow-up time during which the outcome could not have occurred. When it occurs, it usually results in downward bias, as individuals who end up classified as exposed must have survived free of the outcome for a nominal period in order to have received their classification as exposed.

Detection bias

The general problem of detection bias is that diagnosis and treatment of an outcome may vary across levels of exposure.[114] It can arise when a disease is underreported or underdiagnosed,[114] as asthma has been for decades.[115,116] There are multiple potential frameworks within which detection bias can occur, as detailed below.

Detection bias type 1 – Unmasking bias

Unmasking bias or detection signal bias[114,117,118] can arise when an exposure induces symptoms that lead to a search for disease and potential diagnosis of the outcome. This is similar to protopathic bias mentioned earlier – which can arise when underlying preclinical symptoms of the outcome of interest affect the treatment of interest – but the two biases are distinct from one another because unmasking bias occurs based on the extent of searching to detect and diagnose an outcome related to symptoms which were observed *following* treatment. In the context of antibiotics and asthma, this could occur if a patient infected with RSV showed signs of wheeze,[21] but was mistakenly treated with antibiotics for a suspected bacterial infection. If the viral infection were persistent and wheeze symptoms progressed,[41] then the antibiotic would likely be recognized as ineffective – despite remaining in healthcare claims records – and the rule-out diagnosis of bacterial infection would likely be considered untrue or insufficient. As wheeze symptoms may be persistent,[41] the practitioner could prescribe treatment for a diagnosis of asthma; in such a scenario, the record of an antibiotic prescription would be an artefact of the practitioner's differential diagnosis method rather than as a cause of asthma.

Detection bias type 2 – Diagnostic suspicion bias

Diagnostic suspicion bias[114,118] can arise when a practitioner's knowledge of a patient's exposure history affects the practitioner's diagnostic process and decision-making. With regard to antibiotics and asthma, this could occur if patients presenting with symptoms indicative of potential (but not certain) asthma and were diagnosed with or without asthma differentially based on practitioners' knowledge of the patient's prior antibiotic exposure. In the proposed study, diagnostic suspicion bias is possible because practitioners can review treatment histories and the

probability of asthma diagnosis could be increased in the exposed because of their awareness of the 'hygiene hypothesis.' This would lead to upward bias.[118]

Detection bias type 3 – Disease reporting bias

Disease reporting bias[114] can arise when a patient's awareness of exposure influences their reporting of symptoms or disease to their practitioner. Not to be confused with recall bias, wherein exposure reporting varies differentially by outcome status, disease reporting bias occurs when outcome classification varies differentially by exposure status. This could occur in studies of infant antibiotic exposure and asthma if a parent were exposed to the 'hygiene hypothesis' or developed other beliefs about antibiotics subsequent to their infant's exposure. If the child experienced respiratory distress, the parent could be more willing to seek care for their child out of concern for potential asthma. Given a similar child with identical respiratory symptoms, another parent with (a) different beliefs about antibiotics or (b) different memory of the child's antibiotic exposure history could have less urgency – or no urgency – to seek care. There are multiple directions in which this bias could manifest, the combination of which would likely lead to minimal impact on inference.

(Other) outcome misclassification bias

Outcome misclassification bias[119,120] can occur when the sensitivity or specificity of the outcome assignment algorithm is imperfect. Outcome classification can be correlated with exposure status or may be independent of it, and the relation between outcome classification and exposure status can have major implications on the characteristics and interpretation of resulting biases. Absolute measures of effect will likely be biased if either sensitivity or specificity is

imperfect, because the true incidence of disease in each exposure group will be incorrectly estimated. However, relative measures can be robust to non-differential outcome misclassification bias if specificity is perfect (i.e., no false positives) – regardless of sensitivity – because misclassification will be proportional across exposure groups. As specificity decreases in such settings, bias increases independently with decreasing outcome prevalence and decreasing sensitivity. In a study of antibiotic exposure and asthma in a large population, outcome misclassification bias of relative effect measures could arise if a large proportion of individuals without asthma were incorrectly classified as having asthma. This bias would be largely non-differential with respect to antibiotic exposure in early life and would likely lead to bias toward the null; resulting inference could therefore misrepresent true effects as null or attenuated.

2.3. CURRENT EPIDEMIOLOGIC EVIDENCE IS CONFLICTING REGARDING THE POTENTIAL ASSOCIATION BETWEEN EARLY LIFE ANTIBIOTIC EXPOSURE AND INCREASED RISK OF ASTHMA.

Antibiotics are largely effective for treatment of specific bacterial infections and suppurative complications.[32,40,102] Following exposure to antibiotics, however, bacterial flora in the gut are depleted and disrupted.[30] In addition to increasing bacterial resistance at the population level,[32,40,44,99,121,122] unnecessary antibiotic use during infancy and childhood may be harmful to children and increase their risk of atopic immune response and asthma.[14,32,123]

Several epidemiologic studies have assessed the association between early antibiotic use and atopic response broadly defined;[28] some provided evidence for an association[26,124] whereas others provided evidence for no association.[124–127] Studies finding no evidence of such an

association did not control adequately for potential confounding by parental history of atopy[125,126] or frequency of doctor visits during childhood.[124,125]

Over the last 15 years, numerous epidemiologic studies have examined the broad association between childhood antibiotic exposure and asthma;[13,14,128] however, all of these studies were limited by intractable biases, and existing literature provides conflicting evidence of the presence or absence of such an association.[13,14,19,28]

Regarding asthma specifically, results of epidemiologic studies since 1999 have been conflicting and subject to myriad potential biases. With one exception,[129] cross-sectional studies[11,130–135] provided consistent evidence of a deleterious association between antibiotic use and asthma. In the only case-control study on the subject, Martel et al.[93] found evidence of an association (adjusted rate ratio 1.59; 95% CI 1.50–1.68) after adjusting for most potential confounding factors; however, that study was susceptible to multiple forms of detection bias, protopathic bias, recall bias and limited inference due to some strata of covariate cross-classifications containing small counts.

Along with other cross-sectional and case-control studies that likely suffered primarily from recall bias and protopathic bias, many cohort studies have already been conducted to assess the association between early antibiotic exposure and childhood asthma. They have provided evidence of a deleterious association between antibiotic use and asthma[7,26,75,92] as well as no evidence of such an association.[13,124,136–143] None of these studies controlled properly for all known confounding factors, and each one was subject to detection or confounding bias.[7,13,14,26,75,92,93,124,136–143]

Despite its assessment of a well-defined cohort using a large administrative database and otherwise strong methods, one study by Marra et al.[7] found a small association (adjusted hazard

ratio 1.12; 95% CI 1.08–1.16) and report evidence for a dose response by stating that the adjusted hazard ratio for >4 courses during infancy was 1.30 (95% CI 1.20–1.41). It should be noted that the average hazard ratio (HR) for incremental antibiotic course, calculated by hand by me from the publication, was 1.06. This study was unable to control sufficiently for potential confounding bias by family history of asthma, parental smoking or the presence of dust mites, which are some of the most important risk factors for asthma. This uncontrolled confounding could have biased the hazard ratio upward.

In a small cohort (n=424), Su[140] found an association (odds ratio, OR 1.5) for a dose-response relation between infant antibiotic exposure and prevalent or past asthma. This study was susceptible to recall bias (because exposure and outcome information were provided by parent interview) and reverse causality (because incident asthma was not ascertained). This study did suggest that a potentially important control variable to minimize confounding by indication is the number of illness visits to a practitioner.[140]

In a 2011 retrospective ancillary study of the Perinatal Risk of Asthma in Infants of Asthmatic Mothers prospective cohort study in southern New England, Risnes reported a deleterious association for antibiotic exposure in the first 6 months and asthma at 6 years (adjusted odds ratio, aOR 1.52; 95% CI 1.07–2.16), and evidence of a dose-response relation (average OR for incremental antibiotic course, calculated by hand from the publication, was 1.31).[26] It also found that the association was strongest in children without family history of asthma.[26] This study minimized protopathic bias by having theoretically distinct exposure and outcome at-risk periods (i.e., infants diagnosed with asthma before 6 months of age were excluded). To minimize confounding by indication and detection bias, this study also reported similar increased risk of asthma for antibiotic exposure among a subgroup of children who had

no record of lower respiratory tract infection (LRTI) or wheeze during the first year of life (aOR 1.78; 95% CI 1.12–2.81).[144] To assess potential confounding by parental history of asthma, this study reported stratified results, and found an increased risk of asthma for antibiotic exposure among those whose parents had no history of asthma (aOR 1.89; 95% CI 1.00–3.58). Although this was consistent with model-adjusted results from a larger study,[75] it was imprecise and based on a sample of only 47 asthma cases. Unfortunately, because exposure and outcome information was collected from parents when the child was 6 years old, this study was subject to recall bias. If antibiotic exposure were reported differentially by asthma status, then effect estimates could be biased upward away from the null. Additionally, this study did not assess different types of antibiotics; controlled for confounding by LRTI occurring during the first year of life, despite exposure ascertainment ending at 6 months; and did not control for the number of visits to a practitioner.

Family history of immune dysfunction as well as other genetic and environmental characteristics that cluster within the family unit are primary causes of asthma.[14,20,26,36] They are important confounding factors in this setting since they likely impact healthcare seeking behaviors and probability of redeeming antibiotic prescriptions. A recent study[36] controlled for family characteristics by matching asthmatic children with their non-asthmatic siblings, and found evidence of increased risk for asthma after 2 years of age.

All prior studies have estimated treatment effects using traditional outcome modeling adjustment methods, which assume homogeneity of antibiotic exposure effects across strata of the multidimensional covariate space, which is probably not a valid assumption. All prior studies which estimated dose-response relation did so by estimating a relative effect measure for any antibiotic exposure versus none and comparing that to the relative effect measure estimate for a

maximum number (e.g., >4) of courses during infancy to zero courses; such estimates do not translate to potential interventions and are therefore may not be appropriate to guide inference.

2.4. SUMMARY

Asthma is one of the most common chronic diseases in childhood, and poses a significant worldwide burden.[1–3] Childhood asthma prevalence ranges 3-7% in Denmark,[4,5] and approximately 10% of children take prescription medication to treat related symptoms.[6,7] The prevalence of asthma, atopic response, and other allergic conditions has increased in recent decades,[1,2,8–12] especially in industrialized areas,[3,13,14] but reasons for these increases are unclear.[1,2,15–19]

Many causes have been hypothesized to explain observed increases in asthma prevalence.[13,14] Along with genetics,[20,21] environmental factors,[20–24] and viral infection,[20,21,24] the "hygiene hypothesis"[25] may also explain recent increases in the prevalence of childhood asthma, especially in industrialized countries.[14] This controversial[19] hypothesis asserts that adequate microbial exposure is important for developing proper immune response in early life.[19,26] Subsequently, child development in an overly hygienic environment – with lower exposure to microbiota – may induce elevated atopic response in children and elevated risk of the development of asthma.[7,14,27–29]

Antibiotic prescribing is common in children,[32,39] but unnecessary use frequently occurs because bacterial and viral variants of upper respiratory tract infections are often clinically indistinguishable.[40–43] Decreasing unnecessary antibiotic prescribing is a critical feature of clinical and public health approaches to stifle mounting threats of population-level bacterial resistance.[32,40,44–47] In western industrialized countries in particular, increasing advocacy for

rational antibiotic prescribing has been associated with decreases in population-level antibiotic use.[48–51] In addition, pneumococcal conjugate vaccination programs have been associated with decreased risk of acute otitis media and lower respiratory tract infections,[52] two of the most common indications for antibiotics in children.

Given that antibiotics deplete and disrupt bacterial flora in the gut,[30,31] they have long been suspected to cause increased risk of atopic immune response and asthma, particularly in children.[14,32] Over the last two decades, numerous epidemiologic studies have examined the potential association between childhood antibiotic exposure and asthma,[12,14,26,33–37] yielding conflicting results.[13,38] Each prior study has been limited by intractable biases, including confounding by indication or other unmeasured factors, reverse causality, other protopathic bias, and recall bias.

CHAPTER 3: BIRTH COHORT EFFECTS ON ANTIBIOTIC PRESCRIBING DURING INFANCY AMONG CHILDREN BORN IN DENMARK, 2004-2012: A NATIONWIDE POPULATION-BASED COHORT STUDY[1]

3.1. INTRODUCTION

During early life, children are often prescribed antibiotics for bacterial infections including acute otitis media.[32,45,122] Approximately 20%-50% of children's antibiotic prescriptions are used to treat non-bacterial upper respiratory tract infections,[99,100,145] for which they are largely ineffective.[29,32,40,100] In western industrialized countries in particular, increasing advocacy for rational antibiotic prescribing has been associated with decreases in population-level antibiotic use.[48–51] In addition, pneumococcal conjugate vaccination programs have been associated with decreased risk of acute otitis media and lower respiratory tract infections,[52] two of the most common indications for antibiotics in children. Taken together, there is a need to better characterize the changing patterns of antibiotic prescribing in children to inform future studies of antibiotic effectiveness and safety.

Although several studies have assessed trends in early life antibiotic prescribing, they have focused on estimating rates in cross-sectional population samples. Such studies[49,50,145–150] count multiple prescriptions per child and tend to present results for coarsely defined subgroups (*e.g.*, age 0-4 years) or for subgroups defined by exogenous variables (*e.g.*, during the year 2010). Further, studies of *rates* implicitly estimate the frequency with which providers

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prescribe/dispense antibiotics to groups of children. In contrast, studies of *risk* aggregate individual-level data to elucidate which well-defined subgroups redeem prescriptions over specific follow-up intervals. Currently, data are limited regarding cohort effects on the risk of overall and medication-specific antibiotic use during early childhood.

In Denmark, the Danish Health and Medicine Authority's Institute for Rational Pharmacotherapy (IRF), a government institute, issued a nationwide bulletin to general practitioners in April 2007 with guidelines for rational antibiotic prescribing.[47] The bulletin stated that antibiotics nominally affect the duration of acute otitis media infection and do not prevent adverse sequelae (*e.g.*, mastoiditis or recurrent acute otitis media).[47] It recommends that if the decision is made to prescribe antibiotics for acute otitis media, that primary treatment be with penicillin V (a narrow-spectrum antibiotic[81]), and amoxicillin (a broad-spectrum antibiotic[81]) be used only after failure of penicillin V.[47] Following this bulletin, the 7-valent pneumococcal conjugate vaccine (PCV7) was added to the Danish childhood vaccination program on 1 October 2007, and the 13-valent vaccine (PCV13) was phased in starting on 19 April 2010.[53–55] No studies to date have evaluated the impact of these events on antibiotic prescribing in Denmark during the first year of life (henceforth, "infancy").

We characterized overall and medication-specific measures of the risk, rate, and burden of antibiotic prescribing during infancy. Our primary objectives were to estimate birth-month and birth-season cohort effects on antibiotic prescribing during infancy in Denmark, and to examine the potential impacts of the IRF bulletin and the PCV7 and PCV13 vaccination programs on population-level antibiotic prescribing trends across birth cohorts over time.

3.2. METHODS

The study population for this nationwide cohort study included all live births occurring in Denmark from 1 January 2004 to 31 December 2012. Children who survived less than one full day were excluded. We used each child's unique ten-digit personal registration number (CPR-number) to link their individual-level data across multiple registries.

Data from the Medical Birth Registry and Civil Registration System

We identified live births using the Danish Medical Birth Registry (MBR), which contains records of all live births in Denmark since 1973.[151] We included children in the study cohort even if their MBR record did not list a father's CPR number. We excluded children from the cohort in the following circumstances: (1) the CPR number in the MBR for the child or mother could not be linked to the Danish Civil Registration System (CRS), which contains information on vital status and migration[152]; (2) the place of childbirth or residence in Denmark could not be ascertained; (3) the child's sex or date of birth was inconsistent across registries; or (4) a record for a redeemed prescription drug preceded a child's date of birth. We examined the remaining cohort of children from date of birth until death, emigration, or for 365 days.

We grouped children by week, month, season (winter: December-February; spring: March-May; summer: June-August; autumn: September-November), and year of birth. We defined 52 weeks in the year based on 7-day increments. Exceptions to the 7-day definition for the week of birth variable were as follows: (1) February 29 was always grouped into week 9 so that every four years there were 8 days in week #9; and (2) week #52 always contained 8 days (24-31 Dec).

We used data from the MBR to identify characteristics of the mother, pregnancy, and birth event. We ascertained demographic information from the MBR and CRS. After obtaining a historical list of hospitals from the Health Care Classification System[153–155] (http://www.medinfo.dk/sks/brows.php, ftp://filer.sst.dk/filer/sks/data/skscomplete/), we conducted online searches to identify each hospital's municipality and region, and classified each hospital as university-affiliated or not. We assigned geographic locations by municipality and region, and used national census data taken on 1 January 2012 (available from Statistics Denmark, http://www.StatBank.dk/bev22) to classify each municipality with regard to population density (number of residents per square kilometer).

Data on antibiotics

We used the Danish National Health Service Prescription Database to identify all prescriptions redeemed nationwide in community pharmacies and hospital-based outpatient pharmacies from 1 January 2004 to 31 December 2013.[156] We identified antibiotic prescriptions using Anatomic Therapeutic Chemical (ATC) J01 codes, which include antibiotics that are administered for uptake through the circulatory system (as opposed to topically). We classified antibiotics by chemical substance (henceforth, "medication"), and further classified each medication as either broad- or narrow-spectrum using definitions set by Denmark's Statens Serum Institut (http://www.medstat.dk/en, "Groups of Medicines" portal).

Overall and medication-specific antibiotic prescribing (risk, rate, burden)

To describe how overall and medication-specific antibiotic prescribing differed across birth-year cohorts, we examined three measures of antibiotic prescribing: (1) one-year risk of at

least one redeemed antibiotic prescription during infancy, estimated as the complement of the Kaplan-Meier survival function (henceforth, "risk"); (2) incidence rate of redeemed antibiotic prescriptions, allowing for multiple redeemed prescriptions per infant (henceforth, "rate"); and (3) one-year burden of antibiotic prescriptions, based on the number of total days supplied for redeemed antibiotic prescriptions throughout infancy. For rate and burden measures, we computed each medication's share of the overall measure, *i.e.*, the proportion of the total number of prescriptions (for the rate) or days supplied (for the burden) for each drug. We calculated 99% confidence intervals (CIs) for risk (using pointwise intervals at one year of follow-up), rate, and burden estimates, and compared overall and medication-specific measures by birth-year cohort.

Birth-season cohort and time to first redeemed antibiotic prescription during infancy

To assess the impact of birth-season on age at first redeemed antibiotic prescription, we estimated time to first redemption during infancy and compared results across birth-season cohorts. Stratifying by birth-month and birth-season (categorized as described above) in separate analyses, we used age in months as the time scale and first redeemed antibiotic prescription as the event of interest. For each stratum of birth-season or birth-month, we estimated the hazard function for first redeemed antibiotic prescription and the risk function based on the complement of the Kaplan-Meier survival function, which accounted for censoring at death or emigration.

Interrupted time series analysis

To assess changes in the trend of antibiotic prescribing over time, we used segmented linear regression analysis of an interrupted time series data structure.[157–160] Separately for each birth-week cohort, we estimated the one-year risk of redeeming at least one antibiotic

prescription during infancy, redeeming at least one amoxicillin prescription during infancy, and redeeming at least one penicillin V prescription.

In our study, interruptions denoted time points when we hypothesized that a population-level change occurring in Denmark could have altered antibiotic use among infants, depending on whether they were born before or after the interruption. We identified five interruptions, detailed in Table 3.1: (1) the IRF bulletin[47]; (2) the PCV7 "catch-up" vaccination program[53,55]; (3) the standard PCV7 vaccination program[53,55]; (4) the transition from PCV7 to the 13-valent pneumococcal conjugate vaccine (PCV13)[55]; and (5) the time when PCV13 program became predominant.[55] Because the IRF bulletin and PCV7 "catch-up" program occurred within one month, we consolidated them and assigned them to 1 May 2006, thus allowing a one-month lag for dissemination of the bulletin.

To control for confounding by seasonality in the interrupted time series analysis, we used a transformed cosine periodic function[161] with terms for sin($2\pi i$/52 radians) and cos($2\pi i$/52 radians), where i denotes the week of birth during the year, i={1, 2, ..., 52}.[162,163] Our full segmented linear regression model was thus specified as:

$$R_w = \alpha + \beta_0(\textit{time after 1 Jan 2004})_w + \beta_1(\textit{time after 1 May 2006})_w + \beta_2(\textit{time after 1 Jul 2007})_w + \beta_3(\textit{time after 19 Jan 2010})_w + \beta_4(\textit{time after 1 Oct 2010})_w + \beta_5(\sin(2\pi i/52)) + \beta_6(\cos(2\pi i/52)),$$

where the dependent variable, R_w, was the one-year risk of at least one redeemed antibiotic prescription during infancy for children born in week w of the study period, w={1, 2, ..., 468}. The trend estimate for any segment in the time series was equal to the baseline trend estimate ($\hat{\beta}_0$) plus $\hat{\beta}$ estimates for all trend changes at interruptions preceding the segment of interest; *e.g.*, for the third segment (1 Jul 2007 until 19 Jan 2010) the estimate was equal to $\hat{\beta}_0+\hat{\beta}_1+\hat{\beta}_2$. (Please see Appendix A for supplementary methodological detail on the interrupted time series analysis.)

All statistical analyses were conducted using SAS software versions 9.2 and 9.3 (SAS Institute, Inc.; Cary, North Carolina USA). Figures were created using R software version 3.2.1 (R Foundation for Statistical Computing; Vienna, Austria) and Inkscape version 0.91 (www.inkscape.org). This study was approved by the Danish Data Protection Agency (record number 2013-41-1790), the Danish Statens Serum Institut (FSEID-00001450), and the institutional review board at the University of North Carolina at Chapel Hill (study 13-3155).

3.3. RESULTS

The final study population included 561,729 live births in Denmark occurring from 1 January 2004 to 31 December 2012. Table 3.2 reports demographic and birth characteristics of infants in the study population. There were 333,298 infants (59.3% of the total cohort) with at least one redeemed prescription for any drug during their first year, 66% of whom had at least one redeemed antibiotic prescription (n=220,655). Antibiotic prescriptions (n=403,886) accounted for 46% of all drug prescriptions (n=878,641) redeemed during infancy.

Overall and medication-specific antibiotic prescribing

The overall risk of at least one redeemed antibiotic prescription during infancy was 39.5% (99% CI: 39.3, 39.6). Table 3.3 shows the one-year risk of having at least one redeemed antibiotic prescription during infancy, stratified by child and maternal characteristics. Boys were at higher risk than girls, birth-month-specific risk peaked from February through May, and risk decreased across the study period. Maternal smoking during pregnancy was associated with

increased risk, and children born to mothers who requested a cesarean delivery were at elevated risk compared to the rest of the population. Risk was not associated with gestational age at birth or birth weight. Geographically, the Zealand and Southern regions had the highest risk, the Central region had the lowest, and higher population density was associated with lower risk (Figure 3.1).

The overall rate of antibiotic prescribing during infancy was 72 redeemed prescriptions per 100 infant-years of follow-up. The overall burden was 67 daily doses per 10,000 infant-days. Table 3.4 shows the risk, rate, and burden of selected antibiotic medications among children in the study. Out of 22 antibiotic medications prescribed to infants in Denmark during the study period, amoxicillin and penicillin V together accounted for roughly 90% of the prescriptions.

Overall one-year risks and rates decreased across birth-year cohorts (Appendix B); however, the overall burden of antibiotic prescribing remained stable, ranging from 61 to 75 daily doses per 10,000 infant-days. Amoxicillin's share of the overall rate and burden increased across cohorts from 2004 through 2012, while penicillin V's share decreased. Erythromycin was the third most common antibiotic prescribed to infants during the study period, but its share of total antibiotic prescriptions was <0.5% by 2012.

Birth-season cohort and time to first redeemed antibiotic prescription during infancy

Figures 3.2 and 3.3 provide complementary depictions of the relation between birth-season cohort, age, and time to first redeemed antibiotic prescription during infancy. The hazard of a first redeemed antibiotic prescription peaked as infants experienced the months between December and March (Figure 3.2A). Comparing birth-month cohorts as they experience different

sequences of seasons through the first year of life, Figure 3.2B shows variation by birth-month cohort in both the profile of the risk function and the magnitude of the risk at one year.

Figure 3.3 shows hazard and risk as a function of age, collapsing months of birth into four birth-season cohorts and anchoring the x-axis at birth. The hazard of a first redeemed antibiotic prescription increased with age through 12 months and that hazard functions peaked in February, leading to variation in hazard by birth-season (Figure 3.3A). Subsequently, risk profiles through infancy also varied by birth-season cohort, resulting in differences between birth-season cohorts in the relation between age and risk (Figure 3.3B). Infants born in the spring had the lowest risk through 6 months of age (6.5%, 99% CI: 6.3%, 6.6%) and the highest through 12 months (44.8%, 99% CI: 44.5%, 45.2%), whereas infants born in the autumn had the highest risk through 6 months of age (11.5%, 99% CI: 11.2%, 11.7%) and the lowest through 12 months of age (34.2%, 99% CI: 33.9%, 34.6%). Subsequently, interpretation of differences in risk between birth-season cohorts was determined in part by the age at which two pointwise risks were compared.

Interrupted time series analysis

Figure 3.4 and Table 3.5 show risk and trend estimates from our interrupted time series analysis. The overall birth-season-adjusted risk of at least one redeemed antibiotic prescription under our segmented regression model decreased from 40.7% (births in 2004) to 34.6% (births in 2012). For children born from 1 January 2004 until 1 May 2006, there was an increasing trend for amoxicillin prescriptions, but no change for penicillin V. After interruption 1, when the IRF bulletin was published and the PCV7 “catch-up” vaccination program was initiated, risk decreased for prescriptions of both amoxicillin and penicillin V. The trend for amoxicillin began

to rebound after standard enrollment began for the PCV7 program 14 months later, but decreased starting at the PCV13 rollout through the end of the study period. Prescribing of penicillin V changed little for children born after 1 July 2007, but decreased after standard PCV13 enrollment began. Interruption effects were an order of magnitude smaller than birth-season cohort effects.

Our sensitivity analysis demonstrated that adding a 7-month lag affected the trend for amoxicillin but not for penicillin V (Appendix C). The baseline trend for amoxicillin from 1 January 2004 until 30 April 2006 was similar with the lag (0.14% per month; 99% CI: 0.10%, 0.19%) and without the lag (0.17% per month; 99% CI: 0.13%, 0.21%). Without the lag, the primary analysis showed a decreasing trend from 1 May 2006 until 1 July 2007 (-0.22% per month; 99% CI: -0.29%, -0.16%). In contrast, the sensitivity analysis estimated a trend near unity starting before the lag through 1 January 2007 (0.03% per month; 99% CI: -0.13%, 0.18%), followed by a precipitous decreasing trend after the lag through 1 July 2007 (-0.58% per month; 99% CI: -0.79%, -0.37%). Trend interpretation did not change in all other sensitivity analyses (Appendix C).

3.4. DISCUSSION

In this nationwide population-based cohort study from 2004 through 2012, we observed decreases over time in the proportion of infants born in Denmark who received antibiotics during their first year of life, and in the total number of antibiotic prescriptions per infant-year. Yet, over time, those infants who received antibiotic prescriptions received increasing numbers of prescriptions and days supplied. Taken together, the increasing concentration of antibiotic

prescribing in a shrinking proportion of the infant population resulted in little change over time in population-level antibiotic burden. Amoxicillin, a broad-spectrum antibiotic, became increasingly prominent over time, while penicillin V prescribing decreased each year after 2005. In our analysis of time to first redeemed antibiotic prescription, we observed that infants' first antibiotic prescriptions occurred more frequently with increasing age, and during winter. As a result, the association between birth-season and risk of redeeming an antibiotic prescription varied with increasing age. In our interrupted time series analysis, we found that risk of at least one redeemed amoxicillin prescription was dynamic during the study period, with decreasing trends after the IRF bulletin and the PCV7 "catch-up" program, and after PCV13 program initiation.

Strengths and limitations of this study

This study has several strengths. The registry databases facilitated our implementation of a large nationwide population-based cohort study of all children born in Denmark, during this nine-year period when antibiotic prescribing practices were in transition. The tax-supported healthcare system for the entire Danish population includes free access to medical care and partial reimbursement of prescribed medications,[152] leading to minimal disparity in access to healthcare services in our study population. Our study linked individual-level data across multiple registries to jointly assess infants' records of redeemed antibiotic prescriptions and their demographic and other health-related characteristics. Furthermore, the registries that we used for this study contain accurate data on the date and medication type of redeemed antibiotic prescriptions, date of birth, residence, and other variables that we assessed.[151,152,156]

Our estimates for the total birth-season cohort effect on antibiotic prescribing are unlikely to be confounded, since season of birth is not affected by other risk factors for antibiotic use during infancy (*e.g.*, birth order, sex, gestational age at birth).[164] Throughout the study period, there were no changes in population-level characteristics of children born in Denmark; further, no new antibiotic formulations were introduced during the study period, and administration of antibiotic prescriptions to infants did not change. This level of stability limits potential for biased interpretation in the interrupted time series study due to confounding and measurement bias or the effects of co-interventions.[158,165]

This study also has some limitations that should be considered when interpreting results. We lacked data on indication since the large majority of antibiotics prescribed to infants are administered by general practitioners, who are not mandated to record a diagnosis to issue prescriptions.[116] This limited our ability to explore infection trends that might have caused changes in antibiotic prescribing over time or across population subgroups. Second, data on redeemed antibiotic prescriptions did not provide information on medication ingestion[156]; however, our study question and interpretation of data focused on antibiotic *prescribing* as the event (rather than taking the drug), and focused primarily on infants' first redeemed antibiotic prescription. We note limited ability to compare results to a control population in the interrupted time series analysis,[159,165] because the IRF bulletin and PCV programs pertained to the nationwide population of children born in Denmark. Trends over time in antibiotic prescribing are driven by the prevalence and infectiousness of circulating illnesses; although we controlled for seasonality and carefully selected relevant interruptions and their time at onset, our results may be biased by our inability to account for underlying differences in circulating illness from year to year, or other unmeasured temporal influences.

Interpreting results compared with other studies

Our risk and rate estimates corroborate previous findings from substantially smaller studies on the risk and rate of overall antibiotic prescribing among infants in Europe.[139,149] We provide new information on overall and medication-specific patterns of antibiotic prescribing, including the correlation between measures of risk, rate, and burden, and how they changed across nationwide birth-year cohorts in Denmark over a nine-year period.

Prior studies[49,50,146–150] of antibiotic prescribing have focused on estimating rates. Although we described rates (redeemed prescriptions ÷ person-time) and burden (days supplied ÷ person-time), we focused primarily on estimating the risk of redeeming at least one antibiotic prescription during infancy. Risk provides information about the aggregate of individual infants with redeemed antibiotic prescriptions – and those without prescriptions – by posing the research question, "in a well-defined cohort, which children had a redeemed antibiotic prescription in their first year, and when was the first prescription redeemed?" In contrast, rate and burden estimates require additional assumptions[166,167] and tend to shift the focus of the research question to the population level, asking "how many redeemed prescriptions (or days supplied) were prescribed for children in a given population subgroup, defined by calendar time or another characteristic?" We recognize that each of these questions has merit depending on the setting; however, if an investigator wishes to assess individual-level determinants of antibiotic prescribing, then the risk estimator is of primary importance.

Results regarding birth-season effects tracked with disease patterns of acute otitis media, a prominent indication for antibiotic treatment, which peaks in the winter and for children over 6 months old.[122] Only two prior studies[147,148] have considered seasonal differences in antibiotic

prescribing in children, also showing that antibiotic prescribing peaked in winter. However, as those studies focused on estimating the rate, the interpretation of seasonality pertained to time periods of peak *usage* rather than to intrinsically different cohorts of children (*i.e.*, defined by birth-season). Other prominent studies of antibiotic prescribing in children[50,99,139,146,149] have not explicitly assessed seasonal differences.

Using detailed individual-level nationwide data on antibiotic prescribing, our analysis provides new information on risk and time to first redeemed antibiotic prescription, which are important estimators for public health. These new findings on birth-season cohort effects may inform two aspects of future pediatric studies examining antibiotics as the exposure of interest: (1) antibiotic exposure status may differ meaningfully between children born in different seasons, and (2) birth-season differences in age at first antibiotic use may modify the effect – intended or unintended – of antibiotic treatment, given increased vulnerability to both short- and long-term effects of microbial insults in early life.[24,168] Investigators of antibiotic exposure effects should consider these cohort effects on effect modification or confounding bias.

This is the first study to explicitly evaluate the impact of the IRF bulletin (in 2007) and pneumococcal conjugate vaccines (from 2007 onward) on antibiotic prescribing patterns in the infant population of Denmark. It is unclear why the amoxicillin trend rebounded for births after July 2007, following implementation of the PCV7 program. Potential explanations include (1) changes in the prevalence of circulating illnesses from 2007 to 2010, particularly among children born in late 2009 whose elevated risk appears unique compared to the overall trend in the two prior years; (2) a temporary minimum threshold effect[158] in 2007-2008; and (3) limited impact of PCV7 on infection prevention.

The delayed decrease in the trend for amoxicillin after allowing a 7-month lag could result from a stronger lagged (versus immediate) effect of either the IRF bulletin or the PCV7 "catch-up" program, or both. If the lagged decrease were caused by the IRF bulletin, then the bulletin would have affected children born after 1 January 2007 more than those born earlier. This would correspond to a larger effect on infants no older than 3 months old when the bulletin was published compared to infants who were 3-12 months old at that time. Given that risk increased with age during infancy, a discernible impact of the bulletin would likely have occurred some months after its publication. At the same time, a strong lagged effect of the PCV7 "catch-up" program would have been plausible if the regular PCV7 program had been associated with decreasing risk.

This analysis builds on prior studies of antibiotic prescribing in early childhood[50,99,139,146–149] by (1) using granular time scales to assess seasonal and secular trends; (2) examining prescribing in the well-defined infant population of Denmark; (3) assessing birth-season cohort effects on time to first redeemed antibiotic prescription; and (4) invoking quasi-experimental methods to explicitly assess potential changes in prescribing due to population-level policies or events. The birth cohort effects described in this study have implications for design and analysis of future studies of antibiotic safety and effectiveness in children that span multiple birth-season or birth-year cohorts, since these clear differences in antibiotic use may render some subgroups inherently more susceptible to downstream side effects than others.

Conclusions

Children's season of birth impacted both their overall risk of redeeming an antibiotic prescription during infancy and their age at first redeemed antibiotic prescription. Amoxicillin

prescribing was dynamic over the study period, but decreased after distribution of a bulletin on rational antibiotic use in general practice and the rollout of two nationwide pneumococcal vaccination programs. Finally, this study provides new information on the correlation between measures of risk, rate, and burden of antibiotic prescribing in the infant population.

Table 3.1. Interruption time points for hypothesized population-level changes in Denmark related to antibiotic use among infants.

Interruption	Date of publication or rollout	First birth cohort to experience potential interruption effect during infancy (negative lag)	Intended effect and description of interrupting policy/event
IRF bulletin	1 April 2007	1 April 2006	Bulletin to encourage rational antibiotic prescribing in general practice. For antibiotic treatment for acute otitis media, it recommended penicillin V for primary treatment and amoxicillin after treatment failure.[47]
PCV7 "catch-up" program	1 October 2007	1 May 2006	Cost-free enrollment in PCV7 program for children between 3-17 months of age on 1 October 2007. Vaccination intended to reduce incidence of invasive pneumococcal disease, pneumococci-related upper and lower respiratory infection, and transition of pneumococci in the general population. Children in this group who received their first PCV7 vaccination before their first birthday were offered a second PCV7 course after an interval of at least one month, and a third course a minimum of two months after the second. Children who received their first course after their first birthday were offered one additional course at least two months after the first.[53–55]
Standard PCV7 program	1 October 2007	1 July 2007	Cost-free enrollment in PCV7 program for children <3 months of age on 1 October 2007, onward. Children in this group were offered a series of three PCV7 courses at 3, 5, and 12 months of age, concurrent with the DTaP/IPV/Hib vaccination.[53–55]
Transition from PCV7 to PCV13	19 April 2010	19 January 2010	Cost-free enrollment in PCV13 program for children <3 months of age on 19 April 2010, onward; however, the Danish childhood vaccination program recommended using all PCV7 stocks before initiating PCV13 administration. PCV13 dissemination was therefore gradual during 2010.[55]
Standard PCV13 program	1 January 2011	1 October 2010	Cost-free enrollment in PCV13 program for children <3 months of age. After the gradual depletion of PCV7 stocks during 2010, PCV13 utilization became predominant nationwide by 2011.[55]

IRF = Institute for Rational Pharmacotherapy; PCV7 = 7-valent pneumococcal conjugate vaccination program; DTaP/IPV/Hib = Diphtheria, Tetanus, acellular Pertussis, Polio and Haemophilus influenzae type b; PCV13 = 13-valent pneumococcal conjugate vaccination program

Table 3.2. Characteristics of infants born in Denmark, 2004-2012.

	Total study population (N = 561,729)	
	No. or (median)	**% or (IQR)**
Demographics		
Sex of child		
Female	273,839	48.7
Male	287,890	51.3
Mother's age at birth (years)	(31)	(27, 34)
Month of birth		
January	45,744	8.1
February	43,229	7.7
March	47,038	8.4
April	45,113	8.0
May	47,488	8.5
June	48,187	8.6
July	50,811	9.0
August	50,745	9.0
September	48,539	8.6
October	47,197	8.4
November	44,376	7.9
December	43,262	7.7
Year of birth		
2004	64,146	11.4
2005	63,757	11.4
2006	64,669	11.5
2007	63,539	11.3
2008	64,556	11.5
2009	62,485	11.1
2010	63,055	11.2
2011	58,386	10.4
2012	57,136	10.2
Born in university hospital	223,817	39.8
Region of birth		
Capital Region	190,832	34.0
Zealand Region	70,089	12.5
Southern Region	112,140	20.0
Central Region	137,420	24.5
North Region	51,248	9.1
Population density of municipality of residence at birth (residents per km^2)	(177)	(87, 794)
Maternal characteristics		
Parity or birth order (live+still births)		
First pregnancy	246,191	43.8
Second	203,881	36.3
Third	76,312	13.6
Fourth	18,643	3.3
Fifth or more	8,359	1.5
Missing	8,343	

No. of pregnancy visits to GP	(3)	(2, 3)
No. of pregnancy visits to midwife	(5)	(4, 6)
No. of pregnancy visits to specialist	(0)	(0, 0)
Mother's pregravid weight (kg)*	(65)	(59, 75)
Mother's pregravid BMI (kg/m^2)†	(23.0)	(20.8, 26.0)
Pregnancy and birth event		
Singleton pregnancy	537,790	95.7
Maternal smoking during pregnancy		
Did not smoke	470,150	85.7
Smoking, amount unknown	1,542	0.3
Stopped smoking in first trimester	11,346	2.1
Stopped smoking after first trimester	2,702	0.5
Smoking, ≤5 cigarettes/day	19,471	3.5
Smoking, 6-10 cigarettes/day	23,822	4.3
Smoking, 11-20 cigarettes/day	17,286	3.1
Smoking, >20 cigarettes/day	2,463	0.4
Missing	12,947	
Gestational age (weeks) at birth	(40)	(39, 41)
Cesarean delivery for this birth	123,250	21.9
Cesarean upon maternal request	15,695	2.8
Operative vaginal delivery	45,183	8.0
Suture to repair birth injury	210,068	37.4
Birth weight (grams)	(3500)	(3150, 3850)
Newborn transferred to NICU	51,964	9.3
Respiratory aid in NICU	23,754	4.2
Sepsis in child	8,989	1.6
Congenital malformation	40,733	7.3
Died during first year of life	1,073	0.2
Emigrated during first year of life	2,807	0.5

IQR = interquartile range; km = kilometer; GP = general practitioner; kg = kilogram; m = meter; BMI = body mass index; NICU = neonatal intensive-care unit

* 5.2% of children had missing data for maternal pregravid weight.

† 5.9% of children had missing data for maternal pregravid BMI.

Table 3.3. One-year risk of redeeming at least one antibiotic prescription during the first year of life according to selected characteristics of infants born in Denmark, 2004-2012.

	At least one redeemed antibiotic prescription		
	No.	**Risk (%)***	**99% CI**
Overall redeemed antibiotic prescriptions	220,655	39.5	39.3, 39.6
≥1 broad-spectrum antibiotic prescription	147,594	26.3	26.1, 26.4
≥1 narrow-spectrum antibiotic prescription	122,319	21.8	21.7, 21.9
Demographics			
Sex of child			
Female	97,357	35.7	35.5, 36.0
Male	123,298	43.0	42.8, 43.3
Month of birth			
January	17,921	39.4	38.8, 40.0
February	18,397	42.8	42.1, 43.4
March	20,996	44.8	44.2, 45.4
April	20,333	45.3	44.7, 45.9
May	20,976	44.4	43.8, 45.0
June	20,553	42.8	42.3, 43.4
July	20,173	39.9	39.3, 40.4
August	17,731	35.1	34.5, 35.6
September	16,407	33.9	33.4, 34.5
October	16,073	34.2	33.6, 34.8
November	15,293	34.6	34.0, 35.2
December	15,802	36.7	36.1, 37.3
Year of birth			
2004	26,266	41.1	40.6, 41.6
2005	26,281	41.4	40.9, 41.9
2006	27,410	42.6	42.1, 43.1
2007	24,491	38.7	38.2, 39.2
2008	24,980	38.9	38.4, 39.4
2009	24,917	40.1	39.6, 40.6
2010	25,057	39.9	39.4, 40.4
2011	21,455	36.9	36.4, 37.4
2012	19,798	34.8	34.3, 35.4
Region of birth			
Capital Region	73,926	39.0	38.7, 39.3
Zealand Region	31,953	45.7	45.2, 46.2
Southern Region	49,422	44.2	43.9, 44.6
Central Region	45,891	33.5	33.2, 33.8
North Region	19,463	38.1	37.6, 38.6
Maternal characteristics			
Parity or birth order (live+still births)			
First pregnancy	85,570	34.9	34.7, 35.2
Second	88,248	43.4	43.2, 43.7
Third	31,785	41.8	41.4, 42.3
Fourth	7,897	42.5	41.6, 43.5
Fifth or more	3,730	45.0	43.6, 46.4
Missing	3,425		
Pregnancy and birth event			

Maternal smoking during pregnancy			
Did not smoke	180,423	38.6	38.4, 38.7
Smoking, amount unknown	680	44.4	41.2, 47.7
Stopped smoking in first trimester	4,581	40.5	39.4, 41.7
Stopped smoking after first trimester	1,158	43.0	40.6, 45.5
Smoking, ≤5 cigarettes/day	8,602	44.3	43.4, 45.3
Smoking, 6-10 cigarettes/day	10,859	45.8	44.9, 46.6
Smoking, 11-20 cigarettes/day	8,034	46.6	45.6, 47.6
Smoking, >20 cigarettes/day	1,109	45.3	42.8, 47.9
Missing	5,209		
Cesarean upon maternal request	6,956	44.4	43.4, 45.5
Congenital malformation	17,528	43.6	42.9, 44.2

CI = confidence interval

* Risk estimates reflect each subgroup's one-year cumulative incidence of at least one redeemed antibiotic prescription during infancy, based on the complement of the Kaplan-Meier survival function, which accounted for censoring at death or emigration.

Table 3.4. Redeemed antibiotic prescriptions by ATC code among infants born in Denmark, 2004-2012 (N=561,729).

				No. of infants with ≥1 redeemed antibiotic prescription		No. of redeemed antibiotic prescriptions			Days supplied of antibiotic medication		
ATC code	ATC level 5 (medication)	ATC level 4 (subgroup)	Spectrum classification*	No.	Risk (%)†	No.	Rate‡	Share (%)§	Days supplied	Burden‖	Share (%)¶
J01	All antibiotics	All antibiotics	All antibiotics	220,655	39.5	403,886	72	100.0	1,368,589	67	100.0
J01CA04	Amoxicillin	Penicillins, extended spectrum	Broad	144,104	25.8	223,999	40	55.5	844,466	41	61.7
J01CE02	Penicillin V	β-lactamase sensitive penicillins	Narrow	104,609	18.7	133,622	24	33.1	341,838	17	25.0
J01CR02	Amoxicillin clavulanate	Combinations of penicillins	Broad	7,541	1.3	10,694	2	2.6	48,539	2	3.5
J01EA01	Trimethoprim	Trimethoprim, derivatives	Narrow	1,362	0.2	2,555	0	0.6	6,643	0	0.5
J01FA01	Erythromycin	Macrolides	Narrow	14,797	2.6	18,240	3	4.5	79,750	4	5.8
J01FA09	Clarithromycin	Macrolides	Narrow	6,024	1.1	7,074	1	1.8	26,142	1	1.9
J01FA10	Azithromycin	Macrolides	Narrow	3,363	0.6	4,127	1	1.0	8,496	0	0.6

ATC = Anatomical Therapeutic Chemical classification

Data not shown for the following antibiotics because of small numbers: ampicillin, pivampicillin, pivmecillinam, dicloxacillin, flucloxacillin, cefuroxime, meropenem, sulfamethizole, roxithromycin, tobramycin, ciprofloxacin, vancomycin, colistin, fusidic acid, and nitrofurantoin.

* Spectrum classification defined by Danish Register of Medicinal Product Statistics, Statens Serum Institut (http://www.medstat.dk/en).

† Risk estimates reflect medication-specific cumulative incidence of at least one redeemed antibiotic prescriptions for during infancy, based on the complement of the Kaplan-Meier survival function which accounted for censoring at death or emigration; the sum of medication-specific risks exceeds overall antibiotic risk because infants could redeem prescriptions for more than one type of antibiotic medication in their first year.

‡ Rate = no. of redeemed prescriptions per 100 infant-years of follow-up

§ Share of antibiotic rate = (no. of redeemed prescriptions) ÷ (total no. of redeemed prescriptions for all antibiotics)

‖ Population-level antibiotic drug burden = days supply per 10,000 infant-days of follow-up

¶ Share of antibiotic burden = (days supply) ÷ (total days supply for all antibiotics)

Table 3.5. Intercept and trend estimates with 99% confidence intervals (CIs) from segmented linear regression model for the risk (per 100 children, %) of at least one redeemed antibiotic prescription during the first year of life among infants born in Denmark, 2004-2012 (N=561,729).

	Any antibiotic (J01)		Amoxicillin (J01CA04)		Penicillin V (J01CE02)	
Parameter	**Estimate* (%)**	**99% CI**	**Estimate* (%)**	**99% CI**	**Estimate* (%)**	**99% CI**
intercept	40.135	39.310, 41.960	23.490	22.766, 24.214	21.047	20.465, 21.630
$\text{trend}_{\text{01Jan2004\_30Apr2006}}$†	0.090	0.045, 0.135	0.170	0.131, 0.210	-0.006	-0.038, 0.026
$\text{trend}_{\text{01May2006\_30Jun2007}}$†	-0.316	-0.390, -0.241	-0.224	-0.289, -0.159	-0.226	-0.278, -0.173
$\text{trend}_{\text{01July2007\_18Jan2010}}$†	0.066	0.030, 0.102	0.089	0.057, 0.121	0.007	-0.019, 0.032
$\text{trend}_{\text{19Jan2010\_30Sep2010}}$†	-0.198	-0.324, -0.071	-0.317	-0.428, -0.206	-0.021	-0.110, 0.068
$\text{trend}_{\text{01Oct2010\_31Dec2012}}$†	-0.193	-0.243, -0.142	-0.138	-0.182, -0.094	-0.084	-0.120, -0.049
$\sin(2\pi i/52)$	5.521	5.209, 5.833	4.172	3.898, 4.445	2.899	2.679, 3.119
$\cos(2\pi i/52)$	-1.690	-2.000, -1.380	-1.339	-1.610, -1.067	-1.066	-1.284, -0.847

CI = confidence interval; i = week of birth during the year, $i=\{1, 2, ..., 52\}$.

* Estimates correspond to the risk (or change in risk) per 100 children.

† Trend estimates are scaled to month intervals, corresponding to change in linear risk per month of calendar time for births occurring between boundary dates.

Figure 3.1. Geographic variation in population density and risk of redeeming at least one antibiotic prescripiton during the first year of life. (Panel A) Geographic variation in population density (in residents per square kilometer, km^2) by municipality, taken from census data issued for 1 January 2012 (available from Statistics Denmark, http://www.StatBank.dk/bev22). (Panel B) Risk of redeeming at least one antibiotic prescription during the first year of life among infants born in Denmark, 2004-2012. Geographic areas are grouped by municipality (n=98) and region (n=5), assigned based on the location of residence following birth. For births that occurred before the 1 January 2007 reformation of governmental districts into 98 municipalities and 5 regions, geographic data have been harmonized according to the current administrative structure. Artificial gaps separate the North, Central, and Southern regions; the Capital region is detailed in the inset and includes the island of Bornholm (to scale), located 160 km east-southeast of Copenhagen. Each region's capital city is labelled and marked by a diamond.

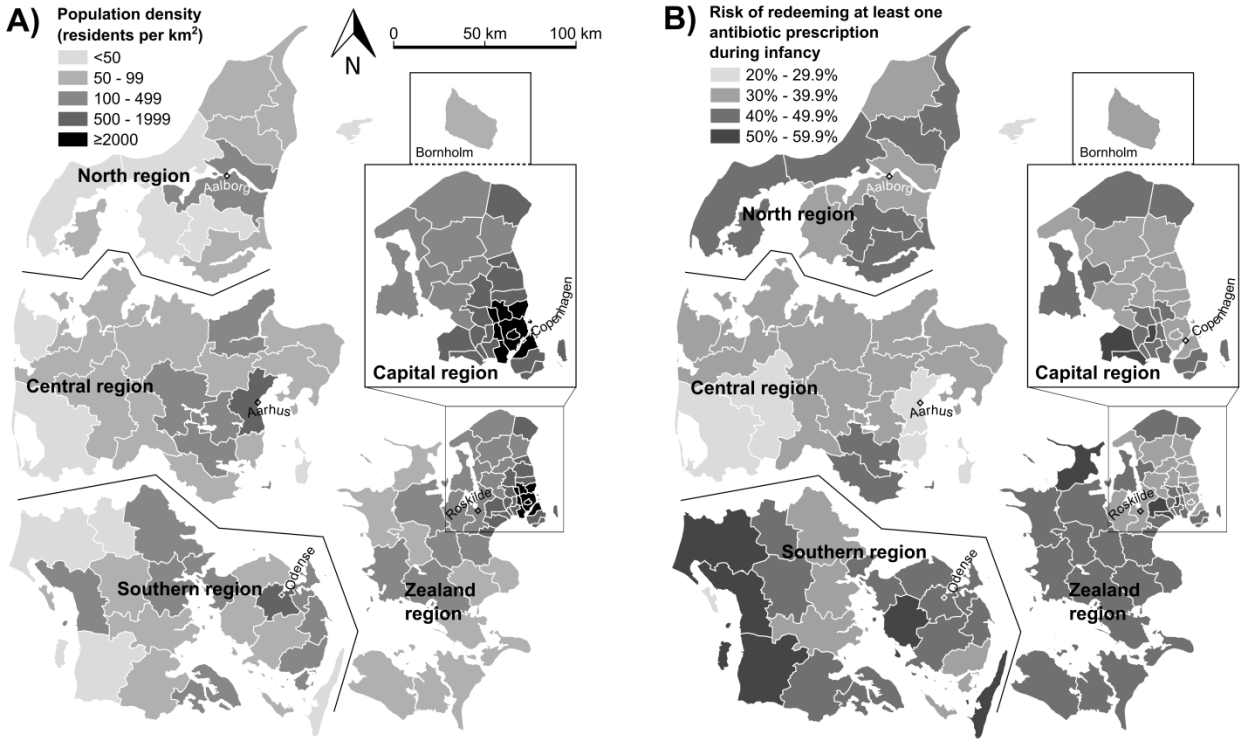


Figure 3.2. Hazard and risk functions for antibiotic prescriptions during the first year of life, stratified by month of birth. (Panel A) Hazard functions for first redeemed antibiotic prescription by month during the first year of life, stratified by month of birth among children born in Denmark, 2004-2012. Each colored curve depicts a birth-month cohort's hazard over one year of follow-up from birth, smoothed using a 7th-order polynomial function with 99% confidence bands (CB). For each month on the calendar time scale, black diamonds show the hazard of a first redeemed antibiotic prescription during that month averaged across all twelve birth-month cohorts; to avoid redundancy, each monthly average hazard is plotted only once. (Panel B) Risk function for at least one redeemed antibiotic prescription during the first year of life by month, stratified by month of birth among children born in Denmark, 2004-2012. The risk function was estimated as the complement of the Kaplan-Meier survival function, with 99% pointwise confidence intervals (CI) taken at each event time (assessed daily).

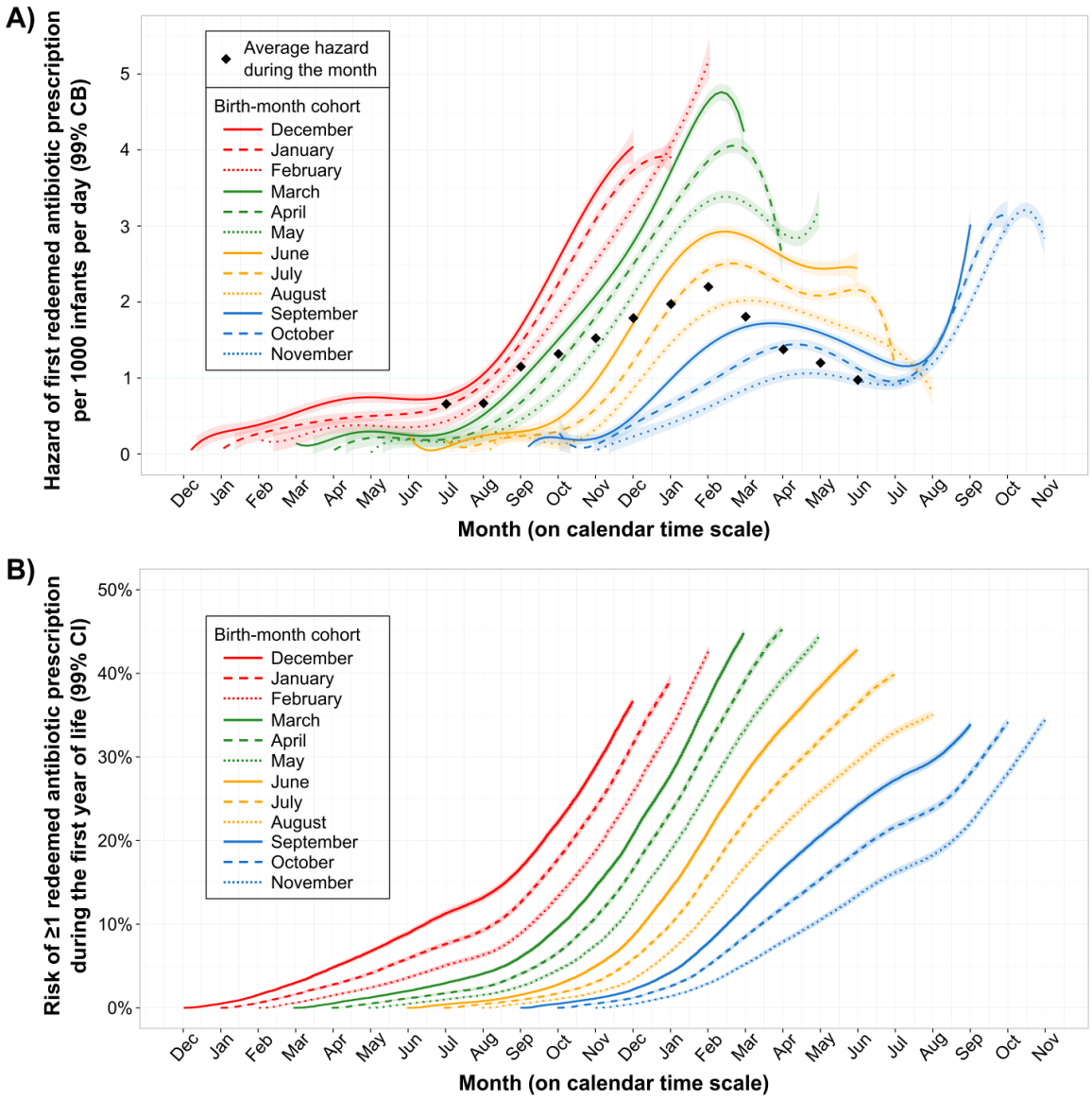


Figure 3.3. Hazard and risk functions for antibiotic prescriptions during the first year of life, stratified by season of birth. (Panel A) Hazard functions for a first redeemed antibiotic prescription by age (in months) and season of birth among children born in Denmark, 2004-2012. Each hazard function is smoothed using a 20th-order polynomial function with 99% confidence bands (CB). Black diamonds represent the average hazard by age in months. (Panel B) Risk function for at least one redeemed antibiotic prescription, by age (in months) through the first year of life, stratified by season of birth, Denmark 2004-2012; the risk function was estimated as the complement of the Kaplan-Meier survival function, with 99% pointwise confidence intervals (CI) taken at each event time (assessed daily). For each month of age, black diamonds show the average hazard of a first redeemed antibiotic prescription (in panel A) and the average risk of at least one redeemed antibiotic prescription (in panel B), collapsed across all birth-season cohorts. Seasons are winter (December-February), spring (March-May), summer (June-August), and autumn (September-November). Shading underneath the x-axis denotes the age interval when each birth-season cohort experienced February, the month when the hazard peaked (see Figure 3.1A). Boundaries for age intervals by birth-season cohort were defined by the 15th of the season's first month to the 15th of the season's last month. For example, children born in the spring (green, March-May) experienced February from 8.5-11.5 months, because children born on May 15 were 8.5 months old on February 1, and children born on March 15 were 11.5 months old on February 28.

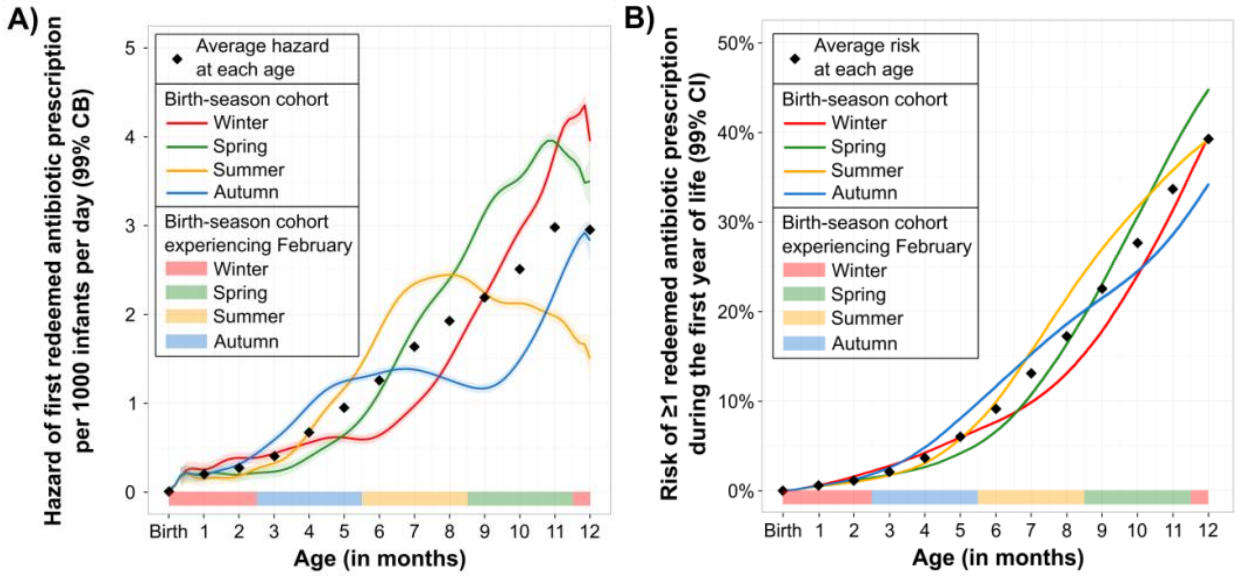
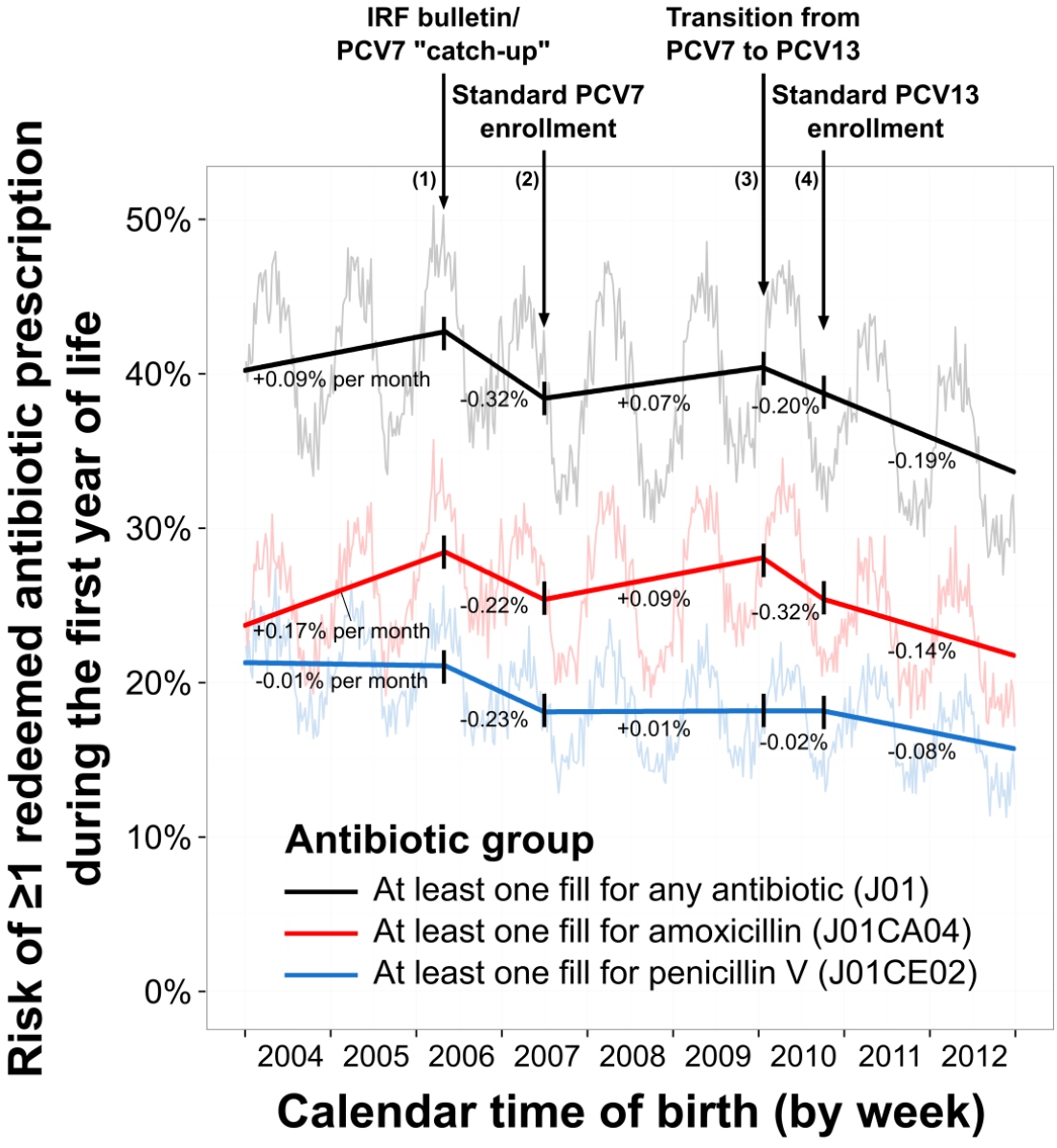


Figure 3.4. Segmented trend lines for the interrupted time series analysis of the risk (%) of at least one redeemed antibiotic prescription during the first year of life for any antibiotic (black), for amoxicillin (red), or for penicillin V (blue), by birth-week cohort, among children born in Denmark during 2004-2012. Interruptions are denoted by downward arrows: (1) the near-coincident Institute for Rational Pharmacotherapy (IRF) bulletin and 7-valent pneumococcal conjugate vaccination (PCV7) "catch-up" enrollment schedule; (2) the standard program for PCV7 enrollment; (3) the nationwide transition from PCV7 to 13-valent pneumococcal conjugate vaccination (PCV13); and (4) the standard program for PCV13 enrollment. Vertical lines crossing a segment indicate interruptions in the time series, when potential changes in the trend for risk were assessed. Segmented trend lines are adjusted for seasonality using a transformed cosine periodic function. For each segment, trend estimates are listed as the change in risk (%) per month.



CHAPTER 4: ANTIBIOTIC PRESCRIBING DURING INFANCY AND RISK OF TREATED OBSTRUCTIVE AIRWAY DISEASES DURING EARLY CHILDHOOD: A REGISTRY-BASED NATIONWIDE COHORT STUDY OF CHILDREN BORN IN DENMARK, 2004-2012[2]

4.1. INTRODUCTION

Asthma is one of the most common chronic diseases in childhood, and poses a significant worldwide burden.[1–3] Persistent asthma in childhood has also been associated with decreased lung function and chronic obstructive pulmonary disease in adulthood.[169] Childhood asthma prevalence ranges 3-7% in Denmark;[4,5] approximately 10% of all children take prescription medication to treat asthma-related symptoms.[6,7]

Antibiotic prescribing is common in children and is a mainstay of treatment for bacterial infections,[32,39] but overprescribing occurs because not all treated infections are bacterial.[40–43] Decreasing unnecessary antibiotic prescribing is central to clinical and public health approaches to stifle mounting threats of bacterial resistance.[44–46] As adequate microbial exposure is hypothesized to be important for developing proper immune response in early life[19,26] and antibiotics deplete and disrupt bacterial flora in the gut,[30,31] they have long been suspected to increase the risk of atopic immune response and asthma, particularly in children.[32,14] Over the last two decades, numerous epidemiologic studies have examined the association between childhood antibiotic exposure and asthma,[12,14,26,33–37] yielding conflicting results.[13,38]

[2] This chapter will be submitted to the *American Journal of Epidemiology*.

Discrepancies between studies are likely a function of residual confounding by indication or other unmeasured factors, reverse causality or recall bias.

Our objective was to examine the association between antibiotic exposure during the first year of life (henceforth, infancy) and incidence of treated airway diseases during early childhood, a proxy for asthma, in Denmark by using a variety of study design and analysis strategies to minimize the potential for bias. We estimated 1-, 2-, and 3-year risk differences using two distinct but complementary approaches – propensity scores (PS)[56,57] and instrumental variables (IV).[58–60] We assessed the dose-response and heterogeneity of antibiotic effects by age, and compared results of the population average effect of antibiotic exposure from both approaches.[20,170]

4.2. METHODS

The study population for this nationwide cohort study included all children born in Denmark from 1 January 2004 to 31 December 2012, identified using the Danish Medical Birth Registry (MBR), which contains all live birth records in Denmark since 1973.[151] We used each child's unique personal registration number (CPR number) to link data across multiple registries, including data on their mother, father, and older siblings sharing the same mother. We excluded children from the study if (1) the CPR number in MBR for the child, mother, or father could not be linked to the Danish Civil Registration System (CRS),[152] (2) the place of childbirth or residence unknown; (3) the child's sex or date of birth was inconsistent across registries, or (4) the child's date of birth was later than a redeemed prescription record.

Data on antibiotic exposure

To ascertain antibiotic exposure during the first year of life, we used the Danish National Health Service Prescription Database (NHSPD) to identify prescriptions for systemic antibiotics redeemed in community pharmacies and hospital-based outpatient pharmacies in Denmark,[156] and classified each prescription by medication (*e.g.*, amoxicillin, penicillin V) (see Appendix D). We calculated age at first redeemed antibiotic prescription, and counted each child's total number of redeemed antibiotic prescriptions during infancy.

Data on treated airway diseases

The 'treated airway diseases' outcome was motivated by our interest in effects on clinically relevant asthma-related symptoms, which often occur before children have reached an age when asthma can be reliably diagnosed.[170,20] Follow-up for the outcome started on the child's second birthday, one year after exposure ascertainment ended, to establish exposure-outcome temporality and reduce potential for reverse causality[26,14] or unmasking bias.[114,117,118] We used the NHSPD to identify prescriptions for airway diseases occurring between 2-5 years of age (see Appendix D). We grouped them into three drug classes: (1) inhaled β_2-adrenoreceptor agonists; (2) inhaled glucocorticoids; and (3) leukotriene antagonists. Children were defined as having treated airway diseases if they redeemed at least one prescription from at least two (out of the three) classes of drugs. Fixed combination treatments could count toward either classification, but not both.

The primary outcome occurrence measures were the risk of treated airway diseases by age 3 (1-year risk), age 4 (2-year risk), and age 5 (3-year risk). Due to administrative censoring

on 31 Dec 2015, estimates of the 2- and 3-year risk were based only on children born during 2004-2011 and 2004-2010, respectively.

Data on covariates

We identified covariates based on previous literature, ascertaining data from the MBR, CRS, NHSPD, Danish National Registry of Patients (NPR), and nationwide municipality-level census.[171] Measured covariate constructs included: demographic characteristics; characteristics of pregnancy, birth, and the perinatal period; family history of disease and healthcare services utilization; and the child's history of illness during infancy. We used sensitivity analysis to assess the influence of controlling for the child's history of illness during infancy, since timing of those covariate measures could occur after antibiotic exposure. See Appendix D for a comprehensive description of data sources and administrative codes for all variables used in the analysis.

Out of 86 measured covariates, eight had missing data; 10.5% of children were missing at least one covariate value, and 1.2% were missing more than two. We used the expectation-maximization algorithm to impute maximum likelihood estimates of individual missing values for these variables,[172,173] based on the assumption of missing data at random conditional on the joint distribution of those eight variables and 76 other non-collinear numeric variables, including antibiotic exposure during infancy and treated airway diseases by age 3.

Propensity score analyses

Our first series of analyses used propensity score (PS) methods to address measured confounding. We examined risk differences for treated airway diseases based on three antibiotic

exposure contrasts during infancy: (1) at least one redeemed antibiotic prescription versus none (henceforth, 'any-versus-none'); (2) at least one redeemed amoxicillin prescription versus at least one redeemed penicillin V prescription and none for either amoxicillin or amoxicillin clavulanate ('any-amoxicillin'); (3) first redeemed antibiotic prescription of amoxicillin versus penicillin V ('first-antibiotic'), for a head-to-head comparison between the two predominant antibiotics in this study population, with differing antibacterial activity.[81] Separately for each contrast, we estimated propensity scores using hierarchically well-formulated[174] logistic regression models containing explanatory terms based on directed acyclic graphs,[175,176] including potential confounding variables,[177] predictors of treated airway diseases,[177] higher-order continuous terms,[178] and multiplicative covariate-covariate interaction terms.[178,179,65]

To estimate 1-, 2-, and 3-year risk differences with 99% confidence intervals (CIs) for effects of antibiotic exposure during infancy on treated airway diseases, we specified weighted linear binomial models[180] with robust variance estimators.[181] To control measured confounding, we balanced measured covariate distributions across exposure using weights based on each child's PS and observed exposure. For the 'any-versus-none' contrast, we used stabilized standardized morbidity ratio (SMR) weights[182–184] to identify the average treatment effect in the treated (ATT). For the 'any-amoxicillin' and 'first-antibiotic' contrasts, we used stabilized inverse-probability-of-treatment (IPT) weights[65,181] to identify the average treatment effect in the population (ATE). To stabilize weights, SMR weights for 'unexposed' children were multiplied by the inverse of the marginal odds of being exposed; IPT weights for all children were multiplied by the prevalence of their observed exposure.[65,181] We conducted asymmetric trimming[63,184,185] at the 99.99th percentile (for the unexposed) and 0.01st percentiles (for the

exposed) of their respective PS distribution to exclude children treated strongly contrary to our prediction.

We assessed the dose-response function using a series of SMR-weighted models to estimate the ATT incremental[186] risk difference for increasing numbers of redeemed antibiotic prescriptions during infancy (1 versus 0, 2 versus 1, 3 versus 2, 4 versus 3, and ≥5 versus 4). For each distinct contrast in the dose-response analysis except '1 versus 0,' SMR weight models included additional explanatory terms for age at first redeemed antibiotic prescription and the type of antibiotic prescribed. We also assessed heterogeneity of risk differences by age at first redeemed antibiotic prescription, restricted to children who were not admitted to neonatal intensive care. We used SMR weights to estimate month-specific ATT risk differences for first antibiotic exposure versus remaining exposure-naïve. For further methodological details on PS-based analyses, see Appendix E.

Instrumental variable analyses

Despite extensive and granular covariate data, some prominent confounding variables remained unmeasured mismeasured. These included paternal smoking, indoor/outdoor air pollution, exposure to dust mites, antibiotic prescription indication, and infant feeding practices. All multivariable methods, including PS, require an assumption of no unmeasured confounding.[59] When suspected unmeasured confounding might lead to notable bias, instrumental variable (IV) approaches might be useful for identifying exposure-outcome effects.[59,60,66,187]

IV analysis is conditional on three assumptions, which in the context of this study are as follows: (1) the instrument affects the proportion of children exposed to antibiotics; (2) the

instrument is unrelated to other covariates such that the association between the instrument and risk of treated airway diseases is not confounded; and (3) the instrument does not directly affect the risk of treated airway diseases. Together, these assumptions imply that the instrument can only be associated with the risk of treated airway diseases if antibiotic exposure has an effect on treated airway diseases.[58,60,68]

We identified two plausible, distinct calendar time-based instruments to contrast otherwise similar birth cohorts, and conducted a separate IV analysis for each. The first analysis used a binary instrument defined by season of birth based on seasonal exposure differences through 12 months of age, and compared children born in March and April (index) versus December and January (referent). Given limited observational data on critical age windows of susceptibility, we also conducted exploratory birth-season IV analyses based on exposure differences through 9 months (July/August versus December/January) and 6 months of age (September versus March).

The second IV analysis used a binary instrument defined by calendar time of birth, comparing birth cohorts from before and after 1 May 2006 that had the largest exposure difference[60] through 12 months of age. This instrument takes advantage of the nationwide rollout of the 7-valent pneumococcal conjugate vaccine (PCV7) for children[54,55] and a nationwide bulletin for general practitioners, which provided guidelines for rational antibiotic prescribing in children.[47] The primary calendar-time IV analysis compared children born from 12 March-29 April 2006 versus 5 March-22 April 2007. All instruments were specified based on analyses of antibiotic prescribing during infancy ignoring outcome data.

We examined all instruments for violation of IV assumptions using information on observed antibiotic exposure and measured covariates, falsification tests for changes in data over

time, and subject-matter expertise.[60,67,188,189] Because instruments were not strong enough to be scaled to a more precise target population,[60] we focused IV estimation on the global average treatment effect in the population (ATE),[190] and calculated non-parametric Balke-Pearl[191] bounds[59,60] on the ATE point estimate. We also estimated risk differences with 99% CIs for the instrument-outcome association, which corresponds to an intention-to-treat (ITT) estimand in a randomized controlled trial with non-compliance.[60] To minimize residual bias,[59] we estimated weighted[192] ITT risk differences with robust variance estimators.[181] In our modelling approach, we fit a logistic regression model for the instrument predicted by the covariates, used predicted probabilities to generate inverse-probability weights for conditional independence between instrument and covariates, and fit a weighted model for the outcome predicted by the instrument. For further methodological details on IV-based analyses, see Appendix F.

All statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Inc.; Cary, North Carolina USA). Figures were created using R software version 3.2.1 (R Foundation for Statistical Computing; Vienna, Austria) and Inkscape version 0.91 (www.inkscape.org). This study was approved by the Danish Data Protection Agency (record number 2013-41-1790), the Danish Statens Serum Institut (FSEID-00001450), and the institutional review board at the University of North Carolina at Chapel Hill (study 13-3155).

4.3. RESULTS

There were 561,729 children born in Denmark during 2004-2012. Before their second birthday, 1,214 of those children died, 5,177 emigrated, and 14,002 had missing data on their father's identity. The final study population thus included of 541,336 children, 96.4% of the

original birth cohort. Death and emigration were rare during outcome follow-up; after their second birthday, 99.2% of children were followed until their fifth birthday.

Overall, 39.6% of children redeemed at least one antibiotic prescription during the first year of life (Table 4.1). The overall risk of treated airway diseases increased from 6.4 per 100 children at 3 years of age to 10.1 per 100 children by 5 years. Due to large sample size, all 99% CI were within 0.1%.

Propensity score analyses

Table 4.1 reports selected covariate characteristics by level of the 'any-versus-none' exposure contrast in observed and SMR-weighted data (see Appendix G for extended table with additional prominent covariates). In observed data, there was notable covariate imbalance between exposed and unexposed children (average standardized absolute mean difference (SAMD)[193] across 83 covariates=0.07, maximum SAMD=0.39). In SMR-weighted data, covariate distributions of the unexposed mimicked the covariate distributions of the exposed (average SAMD=0.003; maximum SAMD=0.012). Across all PS-based analyses, weighting strongly reduced covariate imbalance that existed between exposure groups in observed data (Table 4.2).

In weighted data for the 'any-versus-none' contrast, the 1-, 2-, and 3-year risks of treated airway diseases among the unexposed were 5.8, 8.1, and 9.3 per 100 children; the 1-, 2-, and 3-year risk differences for exposure were 3.3 per 100 children (99% CI: 3.0, 3.6), 4.1 (99% CI: 3.8, 4.4), and 4.5 (99% CI: 4.2, 4.8) (Table 4.3). These estimates suggest an increased risk for treated airway diseases among children with at least one redeemed antibiotic prescription during infancy, compared to none. SMR-weighting increased the referent risk among the unexposed,

resulting in standardized risk difference estimates shifting toward unity compared to the crude. There was a dose-response relation between each antibiotic exposure and risk of treated airway diseases (Figure 4.1). Inverse-variance weighted[194] summary estimates suggest that for each discrete incremental increase in the number of antibiotic prescriptions redeemed during infancy, the standardized 3-year absolute risk of treated airway diseases increased by 2.4 per 100 children (99% CI: 2.3, 2.5).

Among children not admitted to neonatal intensive care (n=492,044), there was some evidence of risk difference heterogeneity by age at first antibiotic exposure in SMR-weighted data (Figure 4.2). Separately for each age, SMR-weighting balanced covariate distributions across exposure groups, subsequently increasing each referent risk. Standardized risk differences increased from 1-4 months, then decreased slightly through the middle of the first year of life, suggesting marginally worse effects when first exposure to antibiotics occurred between approximately 4-7 months. In a sensitivity analysis that omitted control of covariates measured during infancy (*e.g.*, diagnosed infections, visits to pediatrician), risk differences were higher than in the primary analysis, and decreased more markedly from 4-10 months of age (see Appendix H). Covariate imbalance was highest in the first month of life (Table 4.2), suggesting relatively higher residual bias potential in that month for primary and sensitivity analyses.

Compared to the 'any-versus-none' exposure contrast, there was less covariate imbalance in observed data for the 'any-amoxicillin' and 'first-antibiotic' contrasts; imbalances were further diminished in IPT-weighted data (Table 4.2; see detailed tables in Appendix G). In IPT-weighted data, compared to children with at least one redeemed penicillin V prescription during infancy (but none for amoxicillin), children with at least one redeemed amoxicillin prescription

had increased risk of treated airway diseases; there was little evidence of differential risk by type of first antibiotic prescription (Table 4.3).

Instrumental variable analyses

Instruments for birth-season and calendar-time were strong determinants of antibiotic exposure during infancy (Table 4.4). In observed data for all instruments, there was minimal covariate imbalance across levels of the instrument, which was further improved by weighting (Table 4.2). Despite these characteristics, all of these instruments were too weak to identify causal effects, as exemplified by extreme Balke-Pearl bounds (Table 4.3). Further, an exploratory analysis demonstrated high potential for bias and limited precision of local average treatment effect[58,68] estimates based on these instruments (see Appendix I). Weighted ITT estimates did not provide consistent evidence of a causal effect (Table 4.3) and all 99% CIs included unity. Point estimates for birth-season instruments based on exposure differences at 12 and 9 months of age suggested decreased risk of treated airway diseases in populations with higher antibiotic exposure, whereas the 6-month birth-season instrument suggested increased risk. In a sensitivity analysis of the calendar-time instrument (Figure 4.3), to enhance instrument strength, we trimmed two birth-week cohorts from the interior of the birth interval of each instrument level (see Table 4.4). This resulted in weighted ITT estimates that were suggestive of a null instrument-outcome relation (Table 4.3).

4.4. DISCUSSION

In this nationwide cohort study of children born during 2004-2012 in Denmark, we found evidence based on propensity score methods that antibiotic exposure during infancy was associated with increased risk of treated airway diseases from 2-5 years of age. We observed a dose-response relation and evidence suggesting minor heterogeneity of exposure effects by the age at which the first redeemed antibiotic prescription occurred. Head-to-head comparisons of antibiotics demonstrated small, if any, increased risk for exposure to amoxicillin versus penicillin V, which cast some doubt on a causal effect of antibiotics on treated airway diseases, since amoxicillin targets a broader range[81] of bacteria than penicillin V. Unfortunately, the instruments identified for this study were of marginal strength, limiting inference regarding the potential causal relation. Nevertheless, these results also cast some doubt on the presence of a causal effect in this setting.

Results from our propensity score analyses corroborate recent findings[7,26,36,37] suggesting that antibiotic exposure in infancy would increase the risk of airway diseases later in life; however, despite being imprecise, the results from our instrumental variable analysis shed some doubt[35,108] on the causality of the observed association.[14,33,34] Assuming there is an effect, we provide new information on discrete dose-response relations and risk difference heterogeneity by age at first exposure, as well as a head-to-head comparison indicating negligible difference in risk between exposure to amoxicillin versus penicillin V.

A prominent difference across studies of antibiotics and airway diseases is the outcome definition,[34] in part because valid and meaningful measures are difficult to ascertain.[195] Criteria often include asthma diagnosis, medication use, or parental report. The criterion of medication use (alone) has been criticized as an identifier of event occurrence because it does not include

information on asthma diagnosis,[196] yet is widely used.[5,6,195,197,198] Although children under 5 years of age often experience remission of symptoms,[20] airway exacerbations that necessitate treatment pose a serious burden to quality of life for children and their families, and may be a harbinger of chronic conditions like asthma. Furthermore, because general practitioners in Denmark treat most exacerbations but are not mandated to record a diagnosis, the sensitivity (*i.e.*, the proportion of children with airway diseases recognized as such) of our treatment-based outcome definition would be higher than if we had also required a diagnosis code. Higher sensitivity renders the treatment-based outcome definition more optimal for risk difference estimation.[119]

Prior studies offer contradictory evidence of a dose-response relation,[12,14,26,33–37] each one basing inference on a multivariable linear model with categorical or continuous exposure coding. Results from our analysis suggest a deleterious dose-response relation, but are based on a different approach – a series of disjoint models, each one weighted to minimize confounding for that discrete exposure increase. Our approach minimizes potential violation of positivity and exchangeability assumptions,[65,199] and focuses on an estimator that is tied to the clinical decision to prescribe one more antibiotic regimen to a child, or not. These results are subject to potential residual confounding bias similar to the overall 'any-versus-none' exposure contrast.

Three other studies[35–37] have attempted to handle confounding by indication by comparing antibiotics that are classically prescribed for different indications; those studies have found evidence of smaller effects for antibiotics used for skin and urinary infections compared to antibiotics used for respiratory infections. In our analysis of the 'first-antibiotic' contrast, we attempted to address this potential residual bias by comparing antibiotics that are prescribed for similar indications but have different potency. Using an active comparator effectively restricted

the population – based on indication – to children who redeemed a prescription for one of two antibiotics that tend to be prescribed for a similar reason.[184] According to the hygiene hypothesis,[19,25,26] we expected to observe increased risk of treated airway diseases among children first exposed to amoxicillin versus penicillin V[26,75,92] because amoxicillin has a broader spectrum of antibacterial activity.[81] Our findings did not suggest an effect of antibiotic subtype on risk, casting doubt on causality.

Family history of immune dysfunction as well as other genetic and environmental characteristics that cluster within the family unit are primary causes of asthma.[26,14,36,20] They are important confounding factors in this setting since they likely impact healthcare seeking behaviors and probability of redeeming antibiotic prescriptions. Subsequently, along with many other covariates, our study controlled for the parent and older sibling history of obstructive airway diseases, irritable bowel disease, and antibiotic prescriptions; parent and child history of doctor visits; maternal smoking; birth order; and other demographic characteristics. A recent study[36] controlled for family characteristics by matching asthmatic children with their non-asthmatic siblings, and also found evidence of increased risk for asthma after 2 years of age.

Strengths and limitations

This study has several strengths. To our knowledge, it is the largest study conducted to date concerning early life antibiotic exposure and airway diseases in childhood. The registry databases facilitated our implementation of this nationwide cohort study of all children born in Denmark over a nine-year period. The registries that we linked contain accurate data on the date and medication type of redeemed prescriptions, date of birth, residence, data on healthcare utilization, and other variables in this study.[80,152,153,156] The tax-supported healthcare system for

the entire Danish population includes free access to healthcare and partial reimbursement of prescribed medications,[25] leading to minimal disparity in access to healthcare services in our study population.

This is the first study on antibiotics and childhood airway diseases to use propensity scores for controlling measured confounding. In addition to the large study size, propensity scores allowed for more extensive covariate control than prior studies by shifting the burden for model convergence from the treated airway diseases variable onto the antibiotic exposure variable, thus accommodating a larger conditioning set of covariates.[180] Key covariates measured in this study include several that had not been jointly assessed in prior studies, including: parent/older-sibling history of asthma or other obstructive airway diseases; parent/older-sibling history of redeeming antibiotic prescriptions; a granular variable for maternal smoking status; parent/infant history of visits to various clinical specialists; maternal redemption of antibiotic, corticosteroid, and acid-suppressive prescriptions during pregnancy; pregnancy and birth complications; cesarean versus vaginal delivery; birth order; gestational age at birth; birth weight; parental immigrant status; geographic location of child's residence; time-varying population-level pneumococcal vaccination coverage; admission and treatments in neonatal intensive care; and diagnosis during infancy of congenital malformation. As a result of using propensity scores, the ATT/ATE estimands specified in our analyses promote clear inference regarding hypothetical exposure interventions which might have clinical or policy relevance at the population level.

This is also the first study on this topic to use instrumental variables to address confounding bias, which threatens validity of all studies on this topic that rely on measured confounding control. Because available data were lacking information on some key potential

confounding variables, we addressed the possibility of residual unmeasured confounding by using instrumental variable analyses which do not require an assumption of no unmeasured confounding. The findings from our instrumental variable analysis were hampered by weak instruments but nevertheless cast some doubt on causality.

This study also has some important limitations. We lacked data on indication since most antibiotics prescribed to infants are administered by general practitioners who are not mandated to record a diagnosis to issue prescriptions.[39] This limited our ability to control confounding by indication, but we controlled for children's hospital-diagnosed infection history and their frequency of doctor visits during infancy. Two prior studies[35,36] used antibiotic subtype to assess confounding by indication; however, we were precluded from adopting this approach because 90% of antibiotic prescriptions in our study population were for amoxicillin or penicillin V.

The NHSPD lacks information on medication use.[156,200] Our interpretation of data implies a correlation between filling a prescription and subsequently taking the drug as prescribed, which could result in exposure misclassification. This would not affect our outcome, however, because obtaining medication to treat one's child's respiratory symptoms is in itself meaningful, irrespective of whether the child actually used the medication or not. There are myriad potential definitions that could be used to identify outcomes of interest in this context, and we purposefully selected the outcome of treated airway diseases because it is a common, serious condition in early childhood. Further, in an analysis of data on children with follow-up data through 7 years of age, we observed that treated airway diseases by age 5 were predictive of asthma and treatment of related symptoms at age 7 (see Appendix J). Children classified with treated airway diseases by age 5 accounted for approximately 75% of children with asthma at

age 7, and 30% still received treatment for exacerbations at age 7. There was also agreement of treated airway diseases classification across discrete years of age from 2-5 (see Appendix K).

Besides age at first exposure, specifying other sources of potential effect heterogeneity (*e.g.*, sex, birth order, family history of asthma) was beyond the scope of this article. Even in the presence of effect heterogeneity, however, propensity score-based approaches estimate valid marginal exposure effects[183,184] (*i.e.*, the ATT and ATE). Thus, our study is useful for interpreting population average effects in Denmark, but some subgroup-specific effects remain in question.

The instruments identified for this analysis were not strong enough for identifying a meaningful causal estimate. The ITT estimator does not provide optimal inference since it is likely biased toward the null[60] compared to estimates of *exposure*-outcome effects; however, the ITT estimate still addresses potential exposure effects in the total population of children. Given that it is unlikely that other population-based data sources would provide substantially better covariate information to obtain covariate-controlled effect estimates, investigators should aim to identify stronger instruments or other quasi-experimental applications that could identify sufficiently precise causal estimates.

Conclusions

Despite extensive covariate data to control confounding bias in this nationwide cohort study of children born in Denmark during 2004-2012, our propensity score analysis still indicates that antibiotic exposure during infancy is associated with increased risk of treated airway diseases from 2-5 years of age. Two analyses addressing unmeasured and residual confounding (active comparator, IV) showed no association, however, thus increasing doubts about a causal

interpretation. Future research should focus on identifying settings where stronger instrumental variables are available.

In light of inconclusive evidence in this study and in the published literature to date, antibiotic exposure during early life may or may not cause asthma or other related respiratory dysfunction in later childhood. Even if there is no true causal relation, the overall public health message concerning unnecessary antibiotic use shall remain unchanged. Particularly in children, rational antibiotic prescribing is achievable[48–50] and critical for minimizing unnecessary side effects at the individual level, healthcare expenditures for acute clinical consultation,[32] and bacterial resistance at the population level.

Table 4.1. Selected characteristics of infants born in Denmark during 2004-2012, by level of antibiotic exposure during the first year of life ('any-versus-none') in observed data and stabilized standardized morbidity ratio (SMR) weighted data; N=541,336.

	Observed data			Weighted data	
	Exposed	**Unexposed**		**Unexposed**	
	n = 214,256	**n = 327,080**		**mw = 1.005**	
	%	**%**	**SMD**	**%**	**SMD**
Male sex	55.8	48.2	0.15	55.9	0.00
Birth order (mother's live births only)			0.17		0.01
First-born	39.4	47.7		39.1	
Second	41.1	34.8		41.4	
Third	14.8	13.4		14.9	
Fourth	3.5	3.0		3.4	
Fifth or higher	1.3	1.0		1.2	
Year of birth			0.07		0.01
2004-2006	36.4	33.2		36.8	
2007-2009	33.8	34.2		33.7	
2010-2012	29.8	32.5		29.5	
Season of birth			0.16		0.01
Winter (Dec, Jan, Feb)	23.5	23.3		23.8	
Spring (Mar, Apr, May)	28.5	22.8		28.4	
Summer (Jun, Jul, Aug)	26.6	26.9		26.3	
Autumn (Sep, Oct, Nov)	21.4	27.0		21.4	
Population density of municipality of residence at birth, residents per km^2 (median, IQR)	(161, 85-744)	(177, 87-794)	-0.08	(161, 85-744)	0.00
Older siblings, combined rate of antibiotic prescription*†			0.39		0.01
0	43.3	54.3		42.9	
>0-1	20.7	25.7		20.8	
>1-2	18.0	12.6		18.2	
>2	18.0	7.3		18.1	
Any older family member redeemed ≥1 prescription for obstructive airway disease*	37.8	29.9	0.17	37.9	-0.01
Diagnosis of otitis media*	2.4	0.2	0.19	2.2	0.00
Diagnosis of bronchitis, bronchiolitis, RSV pneumonia*	5.1	2.0	0.16	5.1	-0.01
Infant, no. admissions to pediatrician*			0.27		0.01
0	69.1	80.1		69.0	
1	16.6	12.7		16.7	
≥2	14.3	7.2		14.2	
Maternal smoking during pregnancy‡			0.09		0.00
Did not smoke	84.5	87.5		84.4	
Stopped smoking during pregnancy	2.6	2.4		2.6	
Smoking during pregnancy	12.9	10.1		13.0	

SMD = standardized mean difference (mean difference ÷ pooled standard error)
mw = mean weight

IQR = interquartile range
RSV = respiratory syncytial virus
* Ascertained until the child's first birthday.
† Rate = number of prescriptions ÷ person-years of follow-up until the child's first birthday; extreme rates of medication use were imputed with the 99.9th percentile value from their rate-specific distribution.
‡ Ascertained during the 245 days preceding the child's date of birth.

Table 4.2. Summary measures of covariate imbalance in observed and weighted data based on the average standardized absolute mean difference (SAMD), comparing across levels of antibiotic exposure in propensity score (PS)-based analyses, and comparing across levels of the instrument in instrumental variable analyses.

	Average SAMD	
	Observed data	**Weighted data**
PS contrast: Any-versus-none		
Overall analysis	0.07	0.003
Dose-response analysis		
1 versus 0	0.05	0.002
2 versus 1	0.03	0.002
3 versus 2	0.03	0.002
4 versus 3	0.02	0.003
≥5 versus 4	0.05	0.004
Age at first exposure*		
1st month versus later/never	0.07	0.013, 0.011†
2nd month versus later/never	0.11	0.003, 0.003†
3rd month versus later/never	0.11	0.011, 0.003†
4th month versus later/never	0.11	0.008, 0.003†
5th month versus later/never	0.10	0.003, 0.002†
6th month versus later/never	0.09	0.002, 0.002†
7th month versus later/never	0.08	0.002, 0.001†
8th month versus later/never	0.07	0.001, 0.001†
9th month versus later/never	0.06	0.001, 0.001†
10th month versus later/never	0.05	0.001, 0.001†
11th month versus later/never	0.05	0.002, 0.002†
12th month versus later/never	0.05	0.001, 0.001†
PS contrast: Any-amoxicillin	0.03	0.003
PS contrast: First-antibiotic	0.02	0.002
Instrumental variables		
Birth-season, 12 months‡	0.02	0.001
Birth-season, 9 months§	0.02	0.001
Birth-season, 6 months‖	0.02	0.002
Calendar-time, primary¶	0.03	0.003
*Calendar-time, enhanced***	0.03	0.003

SAMD = standardized absolute mean difference (absolute value of the quotient for the mean difference divided by pooled standard error)
PS = propensity scores
* Restricted to children who were never admitted to neonatal intensive care.

† The second value represents the SAMD summary measure from sensitivity analysis, and is based on the revised set of conditioning covariates for that analysis, which do not include characteristics measured during infancy.

‡ Instrument regarding exposure differences at 12 months, comparing children born in March and April versus December and January

§ Instrument regarding exposure differences at 9 months, comparing children born in July and August versus December and January

‖ Instrument regarding exposure differences at 6 months, comparing children born in September versus March

¶ Instrument regarding exposure differences at 12 months, comparing children born during 12 March-29 April 2006 versus 5 March-22 April 2007

** Instrument regarding exposure differences at 12 months, comparing children born during (12/3/2006-18/3/2006, 26/3/2006-1/4/2006, 9/4/2006-29/4/2006) versus (5/3/2007-18/3/2007, 26/3/2007-1/4/2007, 9/4/2007-22/4/2007)

Table 4.3. Summary of results across all propensity score and instrumental variable analyses for the relation between antibiotic exposure during the first year of life and treated airway disease, among children born in Denmark 2004-2012.

	1-year risk difference (by age 3)					2-year risk difference (by age 4)					3-year risk difference (by age 5)				
	Referent risk	**RD***	**99% CI**	**NNTH**	**RR**	**Referent risk**	**RD***	**99% CI**	**NNTH**	**RR**	**Referent risk**	**RD***	**99% CI**	**NNTH**	**RR**
Propensity scores															
Any-versus-none contrast†															
Crude	4.5	4.7	4.5, 4.8	21	2.0	6.4	5.8	5.6, 6.1	17	1.9	7.5	6.4	6.1, 6.6	16	1.9
SMR-weighted for ATT	5.8	3.3	3.0, 3.6	30	1.6	8.1	4.1	3.8, 4.4	24	1.5	9.3	4.5	4.2, 4.8	22	1.5
Any-amoxicillin contrast‡															
Crude	8.2	1.4	1.1, 1.8	71	1.2	11.0	1.8	1.4, 2.2	56	1.2	12.6	2.0	1.5, 2.4	51	1.2
IPT-weighted for ATE	8.4	1.1	0.7, 1.5	91	1.1	11.3	1.3	0.9, 1.8	75	1.1	12.8	1.5	1.0, 2.0	66	1.1
First-antibiotic contrast§															
Crude	9.3	-0.3	-0.6, 0.0	-327	1.0	12.3	-0.3	-0.7, 0.1	-312	1.0	14.0	-0.4	-0.9, 0.0	-249	1.0
IPT-weighted for ATE	9.1	-0.1	-0.4, 0.3	-1302	1.0	12.2	-0.1	-0.5, 0.3	-1222	1.0	13.8	-0.1	-0.6, 0.3	-792	1.0
Instrumental variables															
Birth-season, 12 months‖															
Balke-Pearl bounds for ATE		-69, 77		-1.4, 1.3			-69, 77		-1.4, 1.3			-70, 77		-1.4, 1.3	
Weighted ITT	6.5	-0.2	-0.5, 0.1	-567	1.0	9.0	-0.4	-0.7, 0.0	-275	1.0	10.3	-0.5	-0.9, 0.0	-217	1.0
Birth-season, 9 months¶															
Balke-Pearl bounds for ATE		-61, 85		-1.6, 1.2			-62, 84		-1.6, 1.2			-62, 84		-1.6, 1.2	
Weighted ITT	6.6	-0.3	-0.6, 0.0	-385	1.0	9.0	-0.3	-0.7, 0.1	-338	1.0	10.4	-0.3	-0.7, 0.1	-371	1.0
*Birth-season, 6 months***															
Balke-Pearl bounds for ATE		-56, 92		-1.8, 1.1			-57, 91		-1.8, 1.1			-57, 90		-1.8, 1.1	
Weighted ITT	6.3	0.3	-0.1, 0.8	296	1.1	8.6	0.2	-0.3, 0.7	469	1.0	9.8	0.4	-0.1, 1.0	226	1.0
Calendar-time, primary††															
Balke-Pearl bounds for ATE		-72, 76		-1.4, 1.3			-71, 76		-1.4, 1.3			-71, 76		-1.4, 1.3	
Weighted ITT	6.2	0.0	-1.0, 1.0	6849	1.0	8.4	-0.3	-1.4, 0.9	-388	1.0	9.5	-0.3	-1.5, 1.0	-385	1.0
Calendar-time, enhanced‡‡															
Balke-Pearl bounds for ATE		-71, 76		-1.4, 1.3			-71, 76		-1.4, 1.3			-71, 76		-1.4, 1.3	
Weighted ITT	6.2	0.2	-1.0, 1.5	467	1.0	8.3	0.0	-1.4, 1.4	4878	1.0	9.4	0.1	-1.4, 1.6	858	1.0

Abbreviations and footnotes on following page.

RD = risk difference point estimate
CI = confidence interval
NNTH = number needed to treat to harm (1÷RD)
RR = risk ratio point estimate
SMR = standardized morbidity ratio
ATT = average treatment effect in the index treatment subgroup
IPT = inverse-probability-of-treatment
ATE = average treatment effect in the population
ITT = intention-to-treat

* Risk difference per 100 children

† Exposure contrast between children who redeemed at least one antibiotic prescription during infancy versus none

‡ Exposure contrast between children who during their infancy redeemed at least one amoxicillin prescription versus at least one penicillin V prescription and none for either amoxicillin or amoxicillin clavulanate

§ Exposure contrast between children whose first redeemed antibiotic prescription during infancy was for amoxicillin versus penicillin V

‖ Instrument regarding exposure differences at 12 months, comparing children born in March and April versus December and January

¶ Instrument regarding exposure differences at 9 months, comparing children born in July and August versus December and January

** Instrument regarding exposure differences at 6 months, comparing children born in September versus March

†† Instrument regarding exposure differences at 12 months, comparing children born during 12 March-29 April 2006 versus 5 March-22 April 2007

‡‡ Instrument regarding exposure differences at 12 months, comparing children born during (12/3/2006-18/3/2006, 26/3/2006-1/4/2006, 9/4/2006-29/4/2006) versus (5/3/2007-18/3/2007, 26/3/2007-1/4/2007, 9/4/2007-22/4/2007)

Table 4.4. Strength of instruments related to antibiotic exposure

Instrument	RD* (99% CI)	*F* statistic	Partial r^2
Birth-season, 12 months†	7.0 (6.4, 7.6)	951	0.005
Birth-season, 9 months‡	9.4 (8.9, 9.9)	2546	0.013
Birth-season, 6 months§	6.2 (5.8, 6.7)	1165	0.013
Calendar-time, primary‖	4.6 (2.5, 6.6)	37	0.002
Calendar-time, enhanced¶	6.2 (3.8, 8.6)	49	0.003

RD = risk difference

CI = confidence interval

* Risk difference per 100 children for the relation between the instrument and redeeming at least one antibiotic prescription during infancy.

† Instrument regarding exposure differences at 12 months, comparing children born in March and April versus December and January.

‡ Instrument regarding exposure differences at 9 months, comparing children born in July and August versus December and January.

§ Instrument regarding exposure differences at 6 months, comparing children born in September versus March.

‖ Instrument regarding exposure differences at 12 months, comparing children born during 12 March-29 April 2006 versus 5 March-22 April 2007.

¶ Instrument regarding exposure differences at 12 months, comparing children born during (12/3/2006-18/3/2006, 26/3/2006-1/4/2006, 9/4/2006-29/4/2006) versus (5/3/2007-18/3/2007, 26/3/2007-1/4/2007, 9/4/2007-22/4/2007).

Figure 4.1. Dose-response relations in standardized morbidity ratio (SMR)-weighted data for increasing antibiotic exposure and risk of treated airway diseases among children born in Denmark, 2004-2012. For 1-year (Panel A), 2-year (Panel B), and 3-year (Panel C) follow-up periods for treated airway diseases, risks and risk differences per 100 children are plotted against incremental increases in the number of redeemed antibiotic prescriptions during infancy, with robust 99% confidence intervals (CI). For each contrast, SMR-weighted data represent children remaining in the analysis after asymmetric trimming at the 99.99th percentile (for the referent exposure group) and 0.01st percentiles (for the index exposure group) of the contrast-specific propensity score distribution. Darkened squares represent risks among index exposure group, whitened squares represent the risk among the referent exposure group, and vertical whiskers represent 99% CIs for risk estimates. In settings with overlapping data on risks, point estimates and 99% CIs were horizontally jittered. Risk differences can be seen as the difference between the index and referent risks for each comparison; additionally, risk differences are plotted using black segments to connect point estimates and gray shading to denote pointwise robust 99% CIs for risk differences. For each follow-up interval (*i.e.*, 1-year, 2-year, or 3-year), the inverse-variance weighted summary estimate is listed for the incremental risk difference per 100 children. The dose-response is indicated by the persistent increased risk of treated airway disease for each index exposure versus its referent, across the series of discrete incremental increases in redeemed antibiotic prescriptions during infancy.

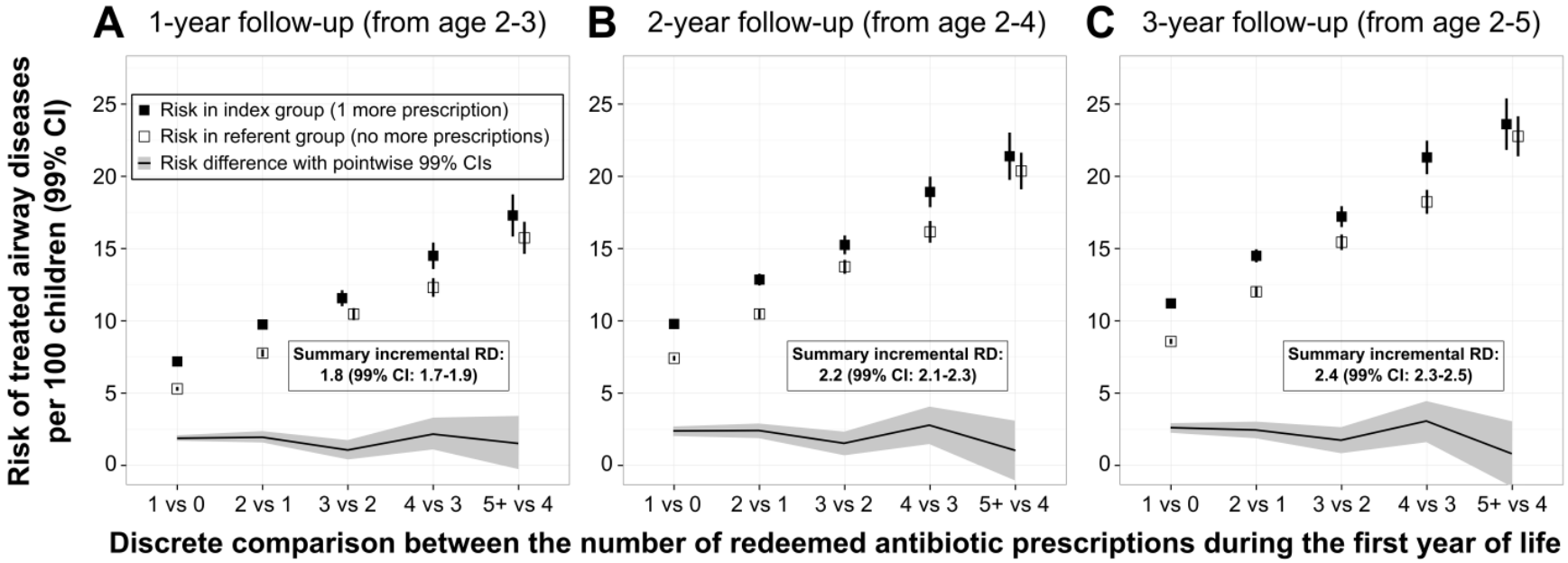


Figure 4.2. Risk difference heterogeneity by age at first redeemed antibiotic prescription for the relation between antibiotic exposure and risk of treated airway diseases among children born in Denmark, 2004-2012, who were never admitted to neonatal intensive care. For 1-year (Panel A), 2-year (Panel B), and 3-year (Panel C) follow-up periods for treated airway diseases, risks for (newly exposed and exposure-naïve children) and risk differences per 100 children are plotted with robust 99% confidence intervals (CI), stratified by age (in months) at which each antibiotic exposure contrast was drawn. For each age-specific contrast, SMR-weighted data represent children who did not redeem an antibiotic prescription prior to the month of age in question, and who remained in the analysis after asymmetric trimming at the 99.99th percentile (for the referent exposure group) and 0.01st percentiles (for the index exposure group) of the age-specific propensity score distribution. Darkened squares represent risks among the newly exposed group, whitened squares represent the risk among the exposure-naïve group, and vertical whiskers represent 99% CIs for risk estimates. In settings with overlapping data on risks, point estimates and 99% CIs were horizontally jittered. Risk differences can be seen as the difference between the index and referent risks for each comparison; additionally, risk differences are plotted using black segments to connect point estimates and gray shading to denote pointwise robust 99% CIs for risk differences.

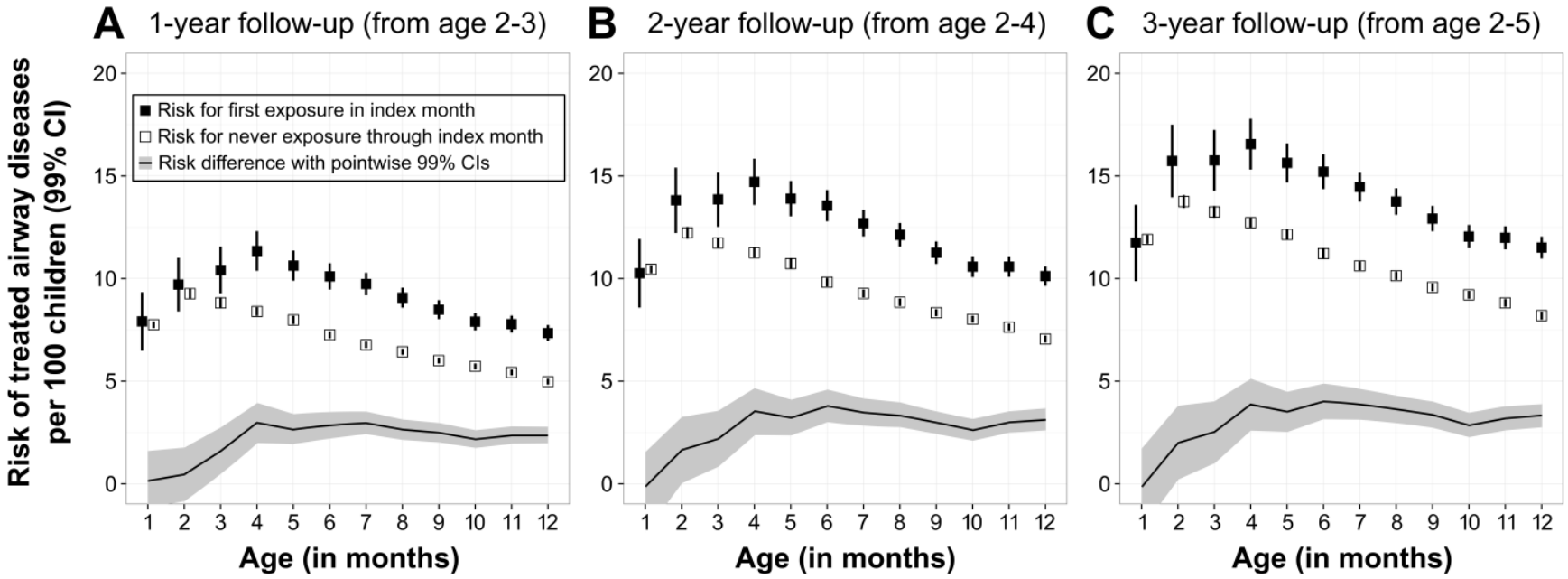
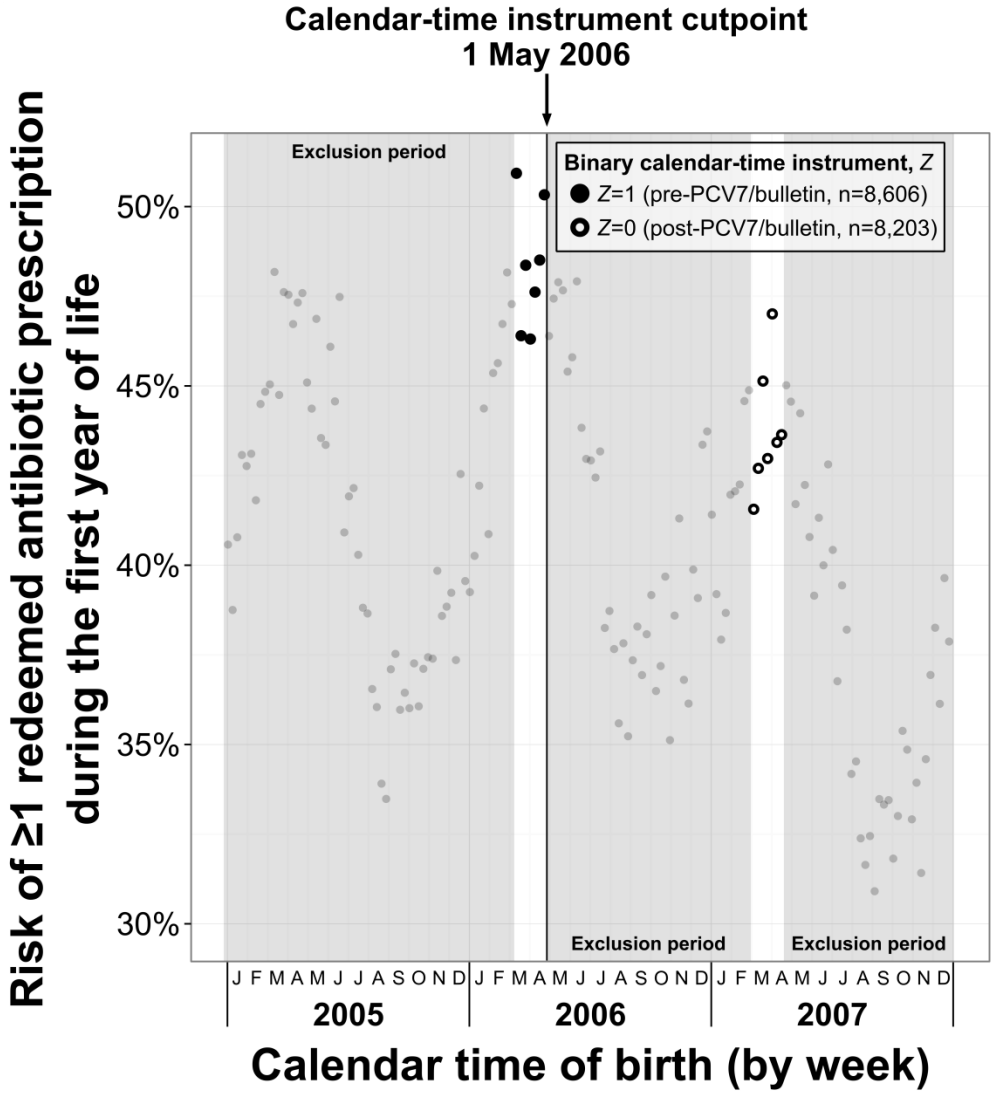


Figure 4.3. Birth cohort differences in the risk of redeeming at least one antibiotic prescription during the first year of life, among children born in Denmark during 2005-2007 (N=185,164). The calendar-time instrument was based on two population-level differences between children born in Denmark before versus after 1 May 2006, which have plausible links to differences in antibiotic use. First, children born after 1 May 2006 were eligible for the Danish childhood vaccination program's 7-valent pneumococcal conjugate vaccine (PCV7);[54,55] children born from 1 November 2006 to 30 June 2007 were enrolled in a three-dose catch-up PCV7 program that had coverage of 71% for the first dose, 67% for the second, and 55% for the third.[54] Second, for children born between 1 May 2006 and 1 May 2007, a nationwide bulletin regarding rational antibiotic use was published during their infancy (in April 2007) by the Danish Health and Medicine Authority's Institute for Rational Pharmacotherapy (IRF), a government institute.[47] To establish conditional independence between the calendar-time instrument and birth-season – and thus reduce potential violation of IV assumptions 2 or 3 – we restricted the calendar-time IV analysis to a comparison of children born in the same season. (Gray shading indicates excluded birth cohorts from the calendar-time instrumental variable analysis. We also limited the time period between[60] the birth-season instrument levels to one year.



CHAPTER 5: DISCUSSION

5.1. SUMMARY OF FINDINGS

The primary objectives for the first specific aim were to estimate birth-month and birth-season cohort effects on antibiotic prescribing during infancy in Denmark, and to examine the potential impacts of the IRF bulletin and the PCV7 and PCV13 vaccination programs on population-level antibiotic prescribing trends across birth cohorts over time. We observed that the proportion of infants born in Denmark who received antibiotics during their first year of life decreased over time, and that the total number of antibiotic prescriptions per infant-year also decreased. Yet, over time, those infants who received antibiotic prescriptions received increasing numbers of prescriptions and days supplied, resulting in little change over time in population-level antibiotic burden. Amoxicillin, a broad-spectrum antibiotic, became increasingly prominent over time, while penicillin V prescribing decreased each year after 2005. In our analysis of time to first redeemed antibiotic prescription, we observed that the association between birth-season and risk of redeeming an antibiotic prescription varied with increasing age. In our interrupted time series analysis, we found that risk of at least one redeemed amoxicillin prescription during infancy decreased after the IRF bulletin and the PCV7 "catch-up" program, and after PCV13 program initiation.

The primary objective for the second specific aim was to examine the association between antibiotic exposure during infancy and incidence of treated airway diseases during early

childhood in Denmark. We found evidence based on propensity score methods that antibiotic exposure during infancy was associated with increased risk of treated airway diseases from 2-5 years of age, as well as a dose-response relation and evidence suggesting minor heterogeneity of exposure effects by the age at which the first redeemed antibiotic prescription occurred. Head-to-head comparisons of antibiotics, however, demonstrated small, if any, increased risk for exposure to amoxicillin versus penicillin V, casting doubt on a causal effect. Unfortunately, the instruments identified for this study were of marginal strength, limiting inference regarding the potential causal relation.

5.2. PUBLIC HEALTH IMPLICATIONS

For the first specific aim, our analysis used detailed individual-level nationwide data on antibiotic prescribing to provide new information on risk and time to first redeemed antibiotic prescription, which are important estimators for public health. The findings regarding birth-season cohort effects may inform two aspects of future pediatric studies examining antibiotics as the exposure of interest. First, there may be inherent differences in antibiotic exposure patterns during early life that are determined in part by season of birth. Second, because of potential differences in vulnerability to both short- and long-term effects of microbial insults in early life based on age at first antibiotic use,[24,168] birth-season differences – which are associated with age at first antibiotic use – may modify the effects of antibiotic exposure on various outcomes. Investigators of antibiotic exposure effects should consider these cohort effects when assessing effect modification or confounding.

This is the first study to date to explicitly evaluate the impact of the IRF bulletin (in 2007) and pneumococcal conjugate vaccines (from 2007 onward) on antibiotic prescribing

patterns in the infant population of Denmark. This study demonstrated that amoxicillin prescribing decreased after the IRF bulletin and the PCV7 "catch-up" program, and after PCV13 program initiation, building on prior studies of antibiotic prescribing in early childhood.[50,99,139,146–149] Future studies of antibiotic safety and effectiveness in children that span multiple birth-season or birth-year cohorts should take into account similar birth cohort effects that may apply to their study setting, since they highlight fundamental differences in antibiotic use that may render some subgroups more susceptible to adverse effects than other subgroups.

In our assessment of the association between antibiotic prescribing and treated airway diseases, propensity score analyses yielded results which aligned with recent evidence[7,26,36,37] suggestive of increased risk of airway diseases later in life following antibiotic exposure during infancy. Assuming there is an effect, we provide new information on discrete dose-response relations and risk difference heterogeneity by age at first exposure. Despite being imprecise, the results from our instrumental variable analysis shed some doubt[35,108] on the causality of the observed association.[14,33,34] We also provide new information on a head-to-head comparison of amoxicillin and penicillin V, which also cast doubt on a causal relation, since amoxicillin exposure was not associated with increased risk of treated airway diseases despite its broader range of antibacterial activity. Our study focused on the adverse outcome of treated airway diseases, which is a relevant outcome for the clinical and public health setting. Although children under 5 years of age often experience remission of symptoms,[20] airway exacerbations that necessitate treatment pose a serious burden to quality of life for children and their families, and may signal chronic conditions like asthma. The symptoms associated with the treated airway diseases outcome are of grave importance in the short-term, and also carry potential to impact long-term health.

5.3. FUTURE RESEARCH

Our propensity score analysis provides evidence suggesting that antibiotic exposure during infancy is associated with increased risk of treated airway diseases from 2-5 years of age, but the head-to-head comparison between amoxicillin and penicillin V casts some doubt on a causal relation. Future research could explore other sources of heterogeneity of the effect of antibiotic exposure on obstructive airway diseases. Other potentially meaningful factors to examine include being of a multiple birth (e.g., twins, triplets), sex, calendar time of birth, family history of asthma and airway diseases, birth order, gestational age at birth, neonatal immune susceptibility/dysfunction, individual pneumococcal vaccination receipt, and exposure to smoking and air pollution in the home. Similarly, future studies could examine comparisons of heterogeneous antibiotic treatment patterns to disentangle the competing effects of age at antibiotic exposure and overall burden of antibiotic exposure on the incidence of childhood asthma and treated airway diseases.

Further clarification of the relation between early life antibiotic exposure and risk of asthma or other obstructive airway diseases is needed, using quasi-experimental analytic methods to identify hypothetical intervention effects in well-defined target populations. In particular, an innovative step toward more conclusive inference could stem from a stronger instrumental variable analysis than ours.

5.4. CONCLUSIONS

Season of birth impacted children's overall 1-year risk of redeeming at least one antibiotic prescription during infancy as well as the age at which their first redeemed antibiotic

prescription occurred. Although penicillin V prescribing declined steadily during 2004-2012, amoxicillin prescribing was dynamic over the study period, decreasing after a bulletin on rational antibiotic use was distributed to general practitioners and two nationwide pneumococcal vaccination programs were rolled out. Birth-season and birth-year cohort effects may be important for assessing effect modification or confounding, and should be considered in future investigations of safety and effectiveness of antibiotic exposures in children.

Our propensity score analysis, despite using extensive covariate data to control confounding, still indicates that antibiotic exposure during infancy is associated with increased risk of treated airway diseases from 2-5 years of age among children born in Denmark during 2004-2012. Analyses that used an active comparator exposure contrast and instrumental variables to address unmeasured and residual confounding showed little evidence of a causal association. Future research should target the identification of stronger instrumental variables to assess the potential causal association between antibiotic exposure in early life and incidence of obstructive airway diseases.

Exposure to antibiotic medication during early life may or may not cause asthma or other related respiratory dysfunction in later childhood, according to inconclusive evidence in this study and current published literature on this topic. Regardless of the true causal relation, however, the overall public health message concerning unnecessary antibiotic use shall remain unchanged. For children in particular, rational antibiotic prescribing is achievable[48–50] and critical for minimizing unnecessary side effects at the individual level, healthcare expenditures for acute clinical consultation,[32] and bacterial resistance at the population level.

APPENDIX A: SUPPLEMENTARY METHODOLOGICAL DETAIL, INTERRUPTED TIME SERIES ANALYSIS.

1. We hypothesized that each interruption would have a gradual effect on the trend of antibiotic prescribing among infants over time. Therefore, we did not include parameters in our model that would measure discontinuity (*i.e.*, changes in level) between adjacent segments.[159,201]
2. Risk measurements corresponded to distinct cohorts of infants so that there was no risk carryover from the same infants being counted in multiple birth-weeks. Therefore our primary analysis did not account for serial autocorrelation of error terms.
3. Overall annual risks were obtained by taking the mean of week-level predicted risks from the model presented in Figure 3.1 for the year of interest.
4. Given the full segmented regression model, as defined previously,

   $R_w = \alpha + \beta_0(\textit{time after 1 Jan 2004})_w + \beta_1(\textit{time after 1 May 2006})_w + \beta_2(\textit{time after 1 Jul 2007})_w + \beta_3(\textit{time after 19 Jan 2010})_w + \beta_4(\textit{time after 1 Oct 2010})_w + \beta_5(\sin(2\pi i/52)) + \beta_6(\cos(2\pi i/52))$

   variables for "time after" an interruption date were coded as 0 if the birth-week *w* occurred before the interruption, and as time (in weeks) since the interruption for birth-weeks occurring afterward. The coefficients of primary interest were:

   α, mean risk for births occurring immediately before January 2004;

   β_0, baseline linear trend for risk before interruptions (1 January 2004 – 30 April 2006);

   β_1, change in trend for risk after the interruption on 1 May 2006;

   β_2, change in trend for risk after the interruption on 1 July 2007;

   β_3, change in trend for risk after the interruption on 19 January 2010;

   β_4, change in trend for risk after the interruption on 1 October 2010.

APPENDIX B: OVERALL AND MEDICATION-SPECIFIC SUMMARIES OF RISK, RATE, AND BURDEN OVER TIME.

Table B.1. Overall and selected medication-specific redeemed antibiotic prescriptions by year of birth in Denmark, 2004-2012 (N=561,729).

RISKS	OVERALL		AMOXICILLIN		PENICILLIN V		ERYTHROMYCIN	
Year of birth	Risk (%)*	99% CI	Risk (%)*	99% CI	Risk (%)*	99% CI	Risk (%)*	99% CI
2004	41.1	40.6, 41.6	24.9	24.5, 25.4	21.1	20.6, 21.5	4.1	3.9, 4.4
2005	41.4	40.9, 41.9	26.0	25.6, 26.5	21.0	20.6, 21.5	3.5	3.3, 3.7
2006	42.6	42.1, 43.1	28.3	27.9, 28.8	20.6	20.2, 21.0	3.6	3.4, 3.7
2007	38.7	38.2, 39.2	25.5	25.1, 26.0	17.9	17.5, 18.3	2.8	2.7, 3.0
2008	38.9	38.4, 39.4	26.0	25.5, 26.4	18.0	17.6, 18.4	2.8	2.6, 2.9
2009	40.1	39.6, 40.6	27.5	27.0, 27.9	18.2	17.8, 18.6	2.6	2.4, 2.7
2010	39.9	39.4, 40.4	27.0	26.6, 27.5	17.3	16.9, 17.8	2.7	2.5, 2.9
2011	36.9	36.4, 37.4	23.7	23.2, 24.1	17.3	16.9, 17.8	1.1	1.0, 1.3
2012	34.8	34.3, 35.4	22.5	22.1, 23.0	16.0	15.6, 16.4	0.2	0.2, 0.3

RATES	OVERALL			AMOXICILLIN			PENICILLIN V			ERYTHROMYCIN		
Year of birth	Rate†	99% CI	Share (%)‡	Rate†	99% CI	Share (%)‡	Rate†	99% CI	Share (%)‡	Rate†	99% CI	Share (%)‡
2004	78	77, 79	100.0	39	38, 39	50.0	28	27, 28	36.0	5	5, 5	6.7
2005	77	77, 78	100.0	41	40, 42	52.9	28	27, 28	35.9	4	4, 5	5.7
2006	81	80, 82	100.0	45	44, 46	55.8	27	26, 27	33.3	4	4, 5	5.5
2007	70	69, 71	100.0	40	39, 40	56.6	23	22, 23	32.5	4	3, 4	5.0
2008	70	70, 71	100.0	40	39, 41	56.7	23	22, 23	32.3	3	3, 4	4.8
2009	73	72, 74	100.0	43	42, 44	58.7	23	22, 23	31.0	3	3, 3	4.3
2010	73	72, 74	100.0	42	41, 43	57.5	22	22, 23	30.5	3	3, 3	4.5
2011	66	65, 67	100.0	36	36, 37	55.2	22	21, 22	32.8	1	1, 1	2.0
2012	60	59, 61	100.0	34	33, 35	56.6	20	19, 20	32.8	0	0, 0	0.4

BURDENS	OVERALL			AMOXICILLIN			PENICILLIN V			ERYTHROMYCIN		
Year of birth	Burden§	99% CI	Share (%)‖	Burden§	99% CI	Share (%)‖	Burden§	99% CI	Share (%)‖	Burden§	99% CI	Share (%)‖
2004	63	63, 64	100.0	36	35, 36	56.1	17	17, 18	27.4	6	6, 6	9.8
2005	65	65, 65	100.0	38	38, 39	59.1	18	17, 18	27.1	5	5, 5	8.1
2006	75	74, 75	100.0	46	46, 46	61.6	19	19, 19	25.7	6	5, 6	7.4
2007	65	64, 65	100.0	40	40, 41	62.1	16	16, 17	25.4	4	4, 4	6.4
2008	65	65, 66	100.0	40	40, 40	61.3	17	16, 17	25.4	4	4, 4	6.1
2009	69	68, 69	100.0	44	44, 44	63.6	16	16, 17	23.9	4	4, 4	5.4
2010	71	71, 72	100.0	45	45, 46	63.2	16	16, 16	22.7	4	4, 4	5.5
2011	67	67, 68	100.0	43	42, 43	63.2	16	16, 16	23.5	2	1, 2	2.3
2012	61	61, 62	100.0	40	40, 41	65.4	14	14, 15	23.4	0	0, 0	0.5

CI = confidence interval

* Risk estimates for at least one redeemed antibiotic prescription for during infancy, using the complement of the Kaplan-Meier survival function which accounted for censoring at death or emigration. The sum of medication-specific risks exceeds overall risk because infants could be prescribed more than one medication in their first year.

† Rate = no. of redeemed prescriptions per 100 infant-years of follow-up

‡ Share of antibiotic rate = (no. of redeemed prescriptions) ÷ (total no. of redeemed prescriptions for all antibiotics)

§ Population-level antibiotic drug burden = days supply per 10,000 infant-days of follow-up

‖ Share of antibiotic burden = (days supplied) ÷ (total days supplied for all antibiotics)

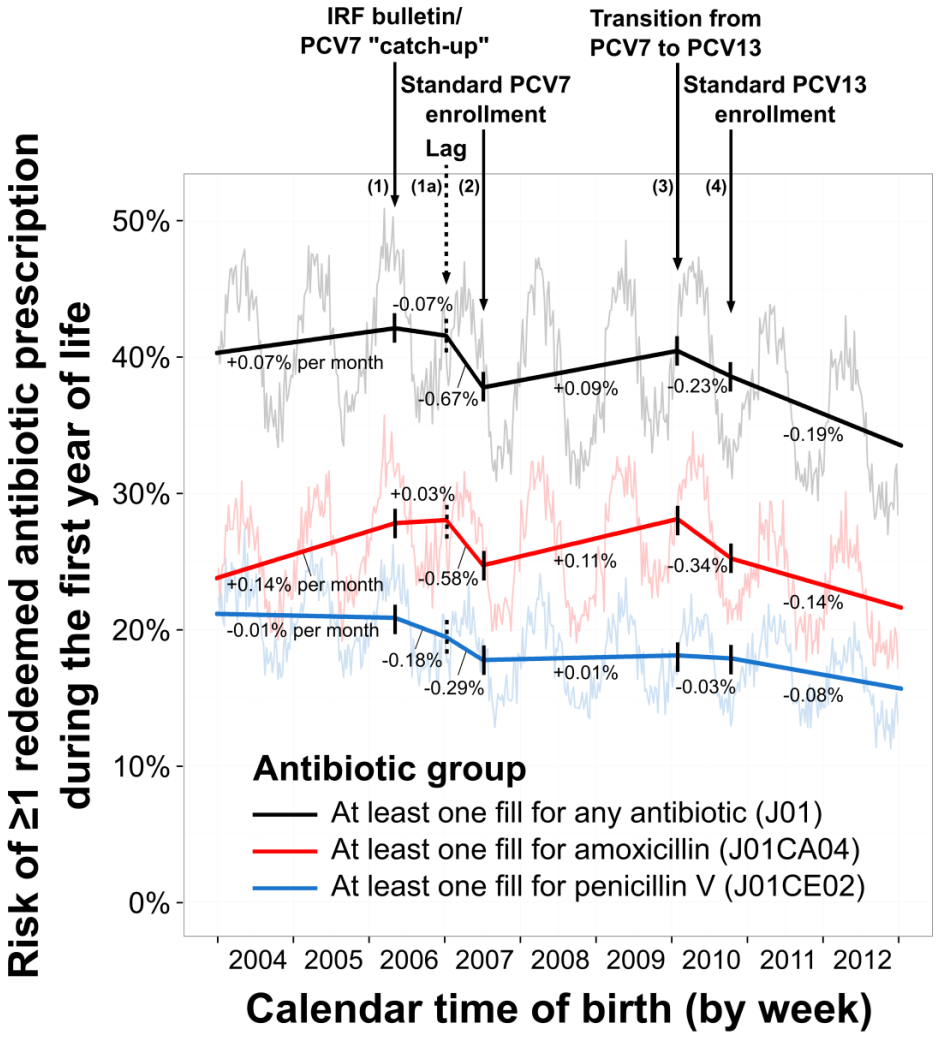
APPENDIX C: SENSITIVITY ANALYSES FOR INTERRUPTED TIME SERIES STUDY

We conducted four sensitivity analyses of the time series study to assess the effects of the number of interruptions we enumerated (analysis 1), how we controlled for seasonality (analyses 2 and 3), and how we accounted for potential serial autocorrelation of error terms (analysis 4). Each sensitivity analysis is described in detail below, and results from these analyses are shown in Figure C.1 and Tables C.1 and C.2.

Sensitivity analysis 1

In the first sensitivity analysis, we added an interruption on 1 January 2007 to relax prior assumptions about (1) the timing of the effects of the IRF bulletin and the PCV7 "catch-up" program and (2) the constancy over time of their effects on antibiotic use. In particular, the IRF bulletin was less current for children born after January 2007 since they were ≤3 months old when the bulletin was published. This sensitivity analysis introduced a new coefficient, β_{1a}, which represented the change in trend after 1 January 2007. After this new interruption, we hypothesized that (1) the bulletin's effect on the trend would be attenuated since children born after January 2007 would have been no more than 3 months old when the bulletin was published, before the vast majority of infants require consideration for their first antibiotic treatment, and (2) the "catch-up" program's effect on the trend would be amplified since increasing numbers of children were enrolled over time. Given that we can only observe the mixture of these two effects, parsing the original second segment into two separate segments was intended to illuminate how their co-occurrence drove time-varying changes in antibiotic use. Figure C.1 shows graphical results from the first sensitivity analysis.

Figure C.1. Graphical results from the sensitivity analysis, which added a fifth interruption to the time series (denoted as 1a) to allow a 7-month lag of the first interruption. The graph shows segmented trend lines for the interrupted time series analysis of the risk of at least one redeemed prescription during the first year of life for any antibiotic (black), for amoxicillin (red), or for penicillin V (blue), by birth-week cohort among children born in Denmark during 2004-2012. Interruptions are denoted by downward arrows: (1) the near-coincident Institute for Rational Pharmacotherapy (IRF) bulletin and 7-valent pneumococcal conjugate vaccination (PCV7) "catch-up" enrollment schedule; (1a) the 7-month lag for the first interruption; (2) the standard program for PCV7 enrollment; (3) the nationwide transition from PCV7 to 13-valent pneumococcal conjugate vaccination (PCV13); and (4) the standard program for PCV13 enrollment. Solid vertical lines crossing a segment indicate interruptions in the time series when potential changes in the trend for risk were assessed; dotted vertical lines indicate interruption 1a, which allowed a 7-month lag for the first interruption. Segmented trend lines are adjusted for seasonality using a transformed cosine periodic function. For each segment, trend estimates are shown as the change in risk (%) per month.



Sensitivity analyses 2, 3, and 4

In the second and third sensitivity analyses, we controlled for seasonality using a vector of 51 birth-week indicator variables, using the first week of the year as the referent (sensitivity analysis 2), and using the cosine function in a two-stage weighted maximum likelihood estimation approach analogous to a weighted least squares approach[162] (sensitivity analysis 3).

In the fourth sensitivity analysis, we conducted the primary analysis and prior three sensitivity analyses using autoregressive parameters to evaluate our assumption that there was no serial autocorrelation of error terms across birth-week cohorts. We assessed serial error autocorrelation between birth-week cohorts using Durbin-Watson test statistics.[202] To account for error autocorrelation in sensitivity analyses, our model for the maximum likelihood estimator of the birth-week-specific risk included the vector of autoregressive parameters that allowed up to a 60-week lag; we used backward elimination to remove autoregressive parameters with a *t*-statistic that was not significant based on an *a priori* type I error level of 0.05.[203]

Summary of Tables C.1 and C.2

For each group of models, the Akaike Information Criterion (AIC) and log likelihood are shown. The AIC provides information on model performance relative to its efficiency, with more negative values indicating relative superiority, and the log likelihood provides information on the goodness of fit of the model. For each series of three models, the first row shows each parameter estimate based on a segmented linear regression model which controls for seasonality using a transformed cosine function (Approach A). The referent for α represents the risk (%), the referent for β_0 represents the trend in risk (%) per month of calendar time, and the referents for β_1, β_2, β_3, *and* β_4 coefficients represent the change in trend at each interruption.

The next two rows in each series show the difference in each parameter for models using Approach B or C compared to the referent model for the series. Plus (+) and minus (-) notation is used to show how estimates differ from the referent estimate for each series. For example, Model #1 estimated a referent risk of 40.1% (α), with an increase in the risk of 0.09% per month from 1 January 2004 to 30 April 2006 (β_0), and a change in the trend of -0.41% per month from 1 May 2006 to 1 July 2007 (β_1). For that series, Approach B (Model #2) differed from Approach A (Model #1) by -1.15% with respect to α, -0.003% with respect to β_0, and +0.004% (shown as 0.00) with respect to β_1.

Models used in the primary analysis are shaded in gray, and graphical results for these models are shown in Figure 3.4 in the main text. The first sensitivity analysis can be reviewed in tabular form by comparing Table C.1 (using four interruptions) to the Table C.2 (which adds interruption 1a), as a comparison between Model #1 and Model #19. The second sensitivity analysis can be reviewed by comparing Approach B to Approach A for any specific setting (*e.g.*, Model #2 versus Model #1). The third sensitivity analysis can be reviewed by comparing Approach C to Approach A for any specific setting (*e.g.*, Model #3 versus Model #1). The fourth sensitivity analysis can be reviewed by comparing the results from models that assume independent error terms to results from autoregressive models for any specific setting (*e.g.*, Model #10 versus Model #1). Results in Tables C.1 and C.2 show that trend changes were robust to multiple specifications that we considered for the linear model.

Table C.1. Model fit criteria and parameter estimates across 4-interruption segmented linear regression models for the risk of at least one redeemed antibiotic prescription among infants born in Denmark, 2004-2012 (N=561,729). Gray shading denotes models used in the primary analysis.

		Model fit criteria		Differences in parameter estimates across models						
	Model #	AIC	LL	α	β_0	β_1	β_{1a}	β_2	β_3	β_4
Analysis of interruptions 1, 2, 3, 4										
Set 1 - Assuming independent error terms										
Any antibiotic										
A*: one-stage, cosine function	1	-2396	1207	ref: 40.13	ref: 0.09	ref: -0.41	—	ref: 0.38	ref: -0.26	ref: 0.01
B†: one-stage, week indicators	2	-2378	1247	-1.15	0.00	0.00	—	0.00	0.00	0.00
C‡: two-stage, cosine function	3	—	—	-0.03	0.00	-0.01	—	0.00	+0.01	-0.01
Amoxicillin										
A*: one-stage, cosine function	4	-2519	1268	ref: 23.49	ref: 0.17	ref: -0.39	—	ref: 0.31	ref: -0.41	ref: 0.18
B†: one-stage, week indicators	5	-2486	1301	-0.10	0.00	0.00	—	0.00	0.00	0.00
C‡: two-stage, cosine function	6	—	—	-0.02	0.00	-0.01	—	0.00	+0.01	-0.01
Penicillin V										
A*: one-stage, cosine function	7	-2722	1370	ref: 21.05	ref: -0.01	ref: -0.22	—	ref: 0.23	ref: -0.03	ref: -0.06
B†: one-stage, week indicators	8	-2710	1413	-0.71	0.00	0.00	—	0.00	0.00	-0.01
C‡: two-stage, cosine function	9	—	—	0.00	0.00	0.00	—	0.00	+0.01	0.00
Set 2 - Using autoregressive parameters										
Any antibiotic										
A*: one-stage, cosine function	10	-2507	1264	ref: 40.16	ref: 0.09	ref: -0.39	—	ref: 0.36	ref: -0.20	ref: -0.06
B†: one-stage, week indicators	11	-2498	1309	-1.01	-0.01	0.00	—	+0.02	-0.06	+0.06
C‡: two-stage, cosine function	12	—	—	-0.04	0.01	-0.02	—	+0.02	-0.05	+0.06
Amoxicillin										
A*: one-stage, cosine function	13	-2622	1325	ref: 23.54	ref: 0.17	ref: -0.39	—	ref: 0.31	ref: -0.39	ref: 0.16
B†: one-stage, week indicators	14	-2615	1372	-0.03	0.00	+0.01	—	-0.01	+0.02	-0.03
C‡: two-stage, cosine function	15	—	—	-0.04	0.00	-0.01	—	+0.01	0.00	+0.01
Penicillin V										
A*: one-stage, cosine function	16	-2769	1400	ref: 21.06	ref: -0.01	ref: -0.21	—	ref: 0.22	ref: 0.00	ref: -0.09
B†: one-stage, week indicators	17	-2751	1439	-0.71	0.00	+0.01	—	0.00	+0.01	-0.01
C‡: two-stage, cosine function	18	—	—	-0.02	0.00	-0.01	—	+0.01	-0.02	+0.02

AIC = Akaike Information Criterion; LL = log likelihood

* Approach A: one-stage maximum likelihood estimation of the risk, controlling for seasonality using a transformed cosine function.

† Approach B: one-stage maximum likelihood estimation of the risk, controlling for seasonality using a vector of 51 birth-week indicator variables.

‡ Approach C: two-stage weighted maximum likelihood estimation of the risk, controlling for seasonality using a transformed cosine function.

Table C.2. Model fit criteria and parameter estimates across 5-interruption segmented linear regression models for the risk of at least one redeemed antibiotic prescription among infants born in Denmark, 2004-2012 (N=561,729).

		Model fit criteria		Differences in parameter estimates across models						
	Model #	AIC	LL	α	β_0	β_1	β_{1a}	β_2	β_3	β_4
Analysis of interruptions 1, 1a, 2, 3, 4										
Set 1 - Assuming independent error terms										
Any antibiotic										
A*: one-stage, cosine function	19	-2409	1214	ref: 40.37	ref: 0.07	ref: -0.13	ref: -0.60	ref: 0.75	ref: -0.31	ref: 0.03
B†: one-stage, week indicators	20	-2395	1257	-1.25	0.00	+0.01	-0.02	+0.01	0.00	0.00
C‡: two-stage, cosine function	21	—	—	+0.01	0.00	+0.01	-0.02	+0.01	+0.01	-0.01
Amoxicillin										
A*: one-stage, cosine function	22	-2537	1279	ref: 23.73	ref: 0.14	ref: -0.12	ref: -0.61	ref: 0.69	ref: -0.46	ref: 0.21
B†: one-stage, week indicators	23	-2508	1313	-0.21	0.00	+0.01	-0.01	+0.01	0.00	0.00
C‡: two-stage, cosine function	24	—	—	+0.01	0.00	0.00	-0.01	0.00	+0.01	-0.01
Penicillin V										
A*: one-stage, cosine function	25	-2721	1371	ref: 21.09	ref: -0.01	ref: -0.17	ref: -0.12	ref: 0.31	ref: -0.04	ref: -0.06
B†: one-stage, week indicators	26	-2710	1414	-0.73	0.00	+0.01	-0.01	+0.01	0.00	0.00
C‡: two-stage, cosine function	27	—	—	+0.01	0.00	+0.01	-0.01	+0.01	0.00	0.00
Set 2 - Using autoregressive parameters										
Any antibiotic										
A*: one-stage, cosine function	28	-2511	1268	ref: 40.55	ref: 0.05	ref: -0.01	ref: -0.82	ref: 0.87	ref: -0.28	ref: -0.01
B†: one-stage, week indicators	29	-2512	1320	-1.17	-0.02	+0.14	-0.26	+0.15	+0.01	-0.02
C‡: two-stage, cosine function	30	—	—	-0.16	+0.01	-0.11	+0.20	-0.10	-0.02	+0.04
Amoxicillin										
A*: one-stage, cosine function	31	-2627	1329	ref: 23.79	ref: 0.14	ref: -0.04	ref: -0.74	ref: 0.76	ref: -0.44	ref: 0.18
B†: one-stage, week indicators	32	-2622	1376	-0.08	-0.01	+0.07	-0.12	+0.07	0.00	-0.01
C‡: two-stage, cosine function	33	—	—	-0.02	+0.01	-0.06	+0.11	-0.06	-0.01	+0.02
Penicillin V										
A*: one-stage, cosine function	34	-2765	1398	ref: 21.15	ref: -0.01	ref: -0.16	ref: -0.10	ref: 0.28	ref: -0.01	ref: -0.08
B†: one-stage, week indicators	35	-2752	1441	-0.75	0.00	0.00	+0.01	-0.01	+0.01	-0.01
C‡: two-stage, cosine function	36	—	—	-0.04	0.00	0.00	-0.03	+0.03	-0.02	+0.02

AIC = Akaike Information Criterion; LL = log likelihood

* Approach A: one-stage maximum likelihood estimation of the risk, controlling for seasonality using a transformed cosine function.

† Approach B: one-stage maximum likelihood estimation of the risk, controlling for seasonality using a vector of 51 birth-week indicator variables.

‡ Approach C: two-stage weighted maximum likelihood estimation of the risk, controlling for seasonality using a transformed cosine function.

APPENDIX D: DATA SOURCES AND DATABASE CODES TO ASCERTAIN EXPOSURE, OUTCOME, AND COVARIATES

Structure of listing for different variable types:

1. Listing structure for variables which required a code to ascertain occurrence:
   - *[Variable]*
     [Data source], [Code Type]: [code 1], [code 2], etc.

2. Listing structure for variables with multiple sub-definitions of drugs or conditions:
   - *[Variable]*
     [Variable sub-definition A]
     [Data source], [Code Type]: [code 1], [code 2], etc.
     [Variable sub-definition B]
     [Data source], [Code Type]: [code 1], [code 2], etc.

3. Listing structure for variables ascertained from database without using additional codes:
   - *[Variable]*
     [Data source], "individual-level value ascertained directly"

4. Listing structure for variables based on admissions to hospital departments:
   - *[Variable]*
     [Data source]

Hospital	Department(s)
[code A]	[code 1], [code 2], etc.

n.b.: i. Database codes were used if they matched code segments preceding 'x' or '.x' below.
ii. Diagnosis codes to inform covariate definition were for primary discharge diagnosis only.
iii. An asterisk (*) denotes that a variable was ascertained using multiple data sources listed.
iv. Abbreviations:

MBR	Danish Medical Birth Registry
CRS	Danish Civil Registration System
NPR	Danish National Registry of Patients
NHSPD	Danish National Health Service Prescription Database
ATC	Anatomic Therapeutic Chemical code in the NHSPD
ICD-8	International Classification of Diseases Eighth Revision; diagnosis codes only, 1977-1993, ascertained from the NPR
ICD-10 (D)	Diagnosis codes, International Classification of Diseases Tenth Revision, 1994- , ascertained from the NPR
ICD-10 (K)	Surgery codes, International Classification of Diseases Tenth Revision, 1996- , ascertained from the NPR
ICD-10 (B)	Treatment codes, International Classification of Diseases Tenth Revision, 1999- , ascertained from the NPR

v. For data on patient visits to clinical specialists, administrative codes for the hospital and department admitting a patient were obtained from NPR data based on codes in the Health Care Classification System[153–155] (available at http://www.medinfo.dk/sks/brows.php and ftp://filer.sst.dk/filer/sks/data/skscomplete/).

Exposure variables – prescriptions redeemed for antibiotic medications

- *Antibiotics for systemic use*
  NHSPD, ATC: J01x

- *Amoxicillin*
  NHSPD, ATC: J01CA04

- *Penicillin V*
  NHSPD, ATC: J01CE02

Outcome variables – obstructive airway diseases

- *Prescriptions redeemed for medications to treat airway diseases*
  Inhaled β_2-adrenoreceptor agonists
  NHSPD, ATC: R03AC02, R03AC03, R03AC04, R03AC12, R03AC13
  Inhaled glucocorticoids
  NHSPD, ATC: R03BA01, R03BA02, R03BA05
  Leukotriene receptor antagonists
  NHSPD, ATC: R03DC03
  Inhaled fixed combinations of glucocorticoids and β_2-adrenoreceptor agonists
  NHSPD, ATC: R03AK06, R03AK07

- *Child asthma diagnosis*
  NPR, ICD-10 (D): J45.0x, J45.1x, J45.2x, J45.8x, J45.9x, J46.9x

Covariates – Group 1 – demographic and family characteristics

- *Child sex*
  MBR, individual-level value ascertained directly

- *Child birth order (mother's live births only)*
  MBR, individual-level value ascertained directly

- *Child date of birth*
  MBR, individual-level value ascertained directly

- *Maternal age at birth*
  MBR, individual-level value ascertained directly

- *Maternal and paternal country of origin*
  CRS, individual-level value ascertained directly

- *Child municipality and region of residence at birth*
  CRS, individual-level value ascertained directly

- *Population density of child municipality of residence at birth*
  National census data taken on 1 January 2012 (available at http://www.StatBank.dk/bev22), individual-level value ascertained directly

- *Family history of redeeming antibiotic prescriptions*
  NHSPD, ATC: J01x

- *Family history of redeeming prescriptions for obstructive airway disease*
  NHSPD, ATC: R03AC02, R03AC03, R03AC04, R03AC12, R03AC13, R03BA01, R03BA02, R03BA05, R03DC03, R03AK06, R03AK07

- *Parental history of asthma diagnosis*
  NPR, ICD-8: 493.x
  NPR, ICD-10 (D): J45.0x, J45.1x, J45.2x, J45.8x, J45.9x, J46.9x

- *Parental history of medical treatment for asthma or other respiratory disorders*
  NPR, ICD-10 (B): GHR0x, GKCx, GFx, GHx

- *Parental history of inflammatory bowel disease*
  NPR, ICD-8: 563.x, 569.04
  NPR, ICD-10 (D): K50.x, K51.x, K52.x

- *Child diagnosis of otitis media during first year of life*
  NPR, ICD-10 (D): H65.x, H66.x, H67.x

- *Child diagnosis of conjunctivitis during first year of life*
  NPR, ICD-10 (D): H10.x, H11.x

- *Child diagnosis of acute upper respiratory infection during first year of life*
  NPR, ICD-10 (D): J00.x, J01.x, J02.x, J03.x, J04.x, J05.x, J06.x

- *Child diagnosis of virus-related lower respiratory diseases during first year of life*
  Acute bronchitis
  NPR, ICD-10 (D): J20.x
  Acute bronchiolitis
  NPR, ICD-10 (D): J21.x
  Bronchitis, unspecified as to acute or chronic
  NPR, ICD-10 (D): J40.x
  Respiratory syncytial virus (RSV) pneumonia
  NPR, ICD-10 (D): J12.1x

- *Child diagnosis of pneumonia during first year of life (excluding RSV pneumonia)*
  NPR, ICD-10 (D): J12.0x, J12.2x, J12.3x, J12.8x, J12.9x, J13.x, J14.x, J15.x, J16.x, J17.x, J18.x

- *Child diagnosis of allergic rhinitis during first year of life*
  NPR, ICD-10 (D): J30.x, J31.x

- *Child diagnosis of atopic dermatitis during first year of life*
  NPR, ICD-10 (D): L20.x, L22.x, L23.x, L27.x

Covariates – Group 2 – parent and child visits to clinical specialists

- *Admission to pulmonology specialists (for parents or child during first year of life)*
  NPR

Hospital	Department(s)
1301	32W
1309	62
1330	521, 52D, 52H, 52L, 52U, 52V
1351	29
1501	32, 04D
1502	06E
2000	211, 213, 21A, 21D, 21F
1549	01
2010	01
2017	02
3800	A0L, D0L, H03, H0L, N03, N0L, R03, R0L, V0L
4202	37
5000	60
5001	05F
5501	054, 05L, 45L
6008	052, 054, 05L
4271	01
6620	11
6630	04F, 30F
6650	33N
7005	05A
7053	01
7062	02
7075	01
7092	01
8001	17, 27x

- *Admission to allergy specialists (for parents or child during first year of life)*
  NPR

Hospital	Department(s)
1301	01Dx, 13x
1501	040x, 044x, 047x, 049x, 04Ex
1549	01x
2017	01x

3800	A0Wx, H0Wx, N0Wx
2514	01x
3026	01x
3523	08x
3528	01x
4202	03x
7053	01x
7075	01x
7092	01x
8001	179x

- *Admission to ear-nose-throat specialists (for parents or child during first year of life)*
  NPR

Hospital	Department(s)
1301	26x
1309	43x
1330	13x
1501	15x
2000	29x
1349	01x
1374	01x
1376	01x
1411	524x, 544x, 554x
1416	01x
1537	01x
1567	017x
2034	01x
3800	E3x, Q0x, S6x, X2x
4202	20x, 21x
5000	30x
5001	11x
5501	083x, 08Hx, 08Nx
6008	12x
6018	01x
6620	19x
6630	08x
6650	41x
7005	12x
7039	01x
7052	01x
7617	01x
7618	01x
8001	22x, 23x
7603	08x, 108x
8034	01x
9001	024x

- *Admission to pediatrician specialists (for child during first year of life)*
  NPR

Hospital	Department(s)
1301	32x, 23Bx
1411	525x, 535x
1330	164x, 60x
1501	04Dx, 18x
1502	17x
1516	37x
2000	10x
1590	01x
3800	B0x, H8x, N9x, V9x
2514	01x
3523	05x
3528	01x
3529	01x
4202	074x, 25x
5000	23x
5001	13x
5501	046x, 04Bx
6007	11x
6006	24x
6620	24x
6630	081x, 09x
6650	24x
7005	15x
8001	25x
8003	16x

Covariates – Group 3 – characteristics of mother and pregnancy

- *Twin or multiple pregnancy*
  MBR, individual-level value ascertained directly

- *Cesarean delivery for any prior birth**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O34.2x, O75.7x, Z35.8Ex

- *Number of prior miscarriages*
  MBR, individual-level value ascertained directly

- *Parity*
  MBR, individual-level value ascertained directly

- *Maternal smoking during pregnancy**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): F17.x, P04.2x, T65.2x, Z35.8M18x, Z39.318x, Z58.7x, Z71.6x, Z72.0x, VRB0x
  NHSPD, ATC: N07BA01, N07BA02, N07BA03, N06AX12, N06AA10, C02AC01, N02CX02, S01EA04, A08AX01

- *Maternal pregravid weight*
  MBR, individual-level value ascertained directly

- *Maternal pregravid body mass index*
  MBR, individual-level value ascertained directly

- *Maternal prescription redemption for inhaled or oral corticosteroid during pregnancy*
  NHSPD, ATC: R03BA01, R03BA02, R03BA05, R03BA07, R03AK06, R03AK07, H02AB04, H02AB06, H02AB07, H02AB09

- *Maternal acid-suppressive drug use during child's pregnancy*
  NHSPD, ATC: A02B.x

- *Maternal diagnosis of high-risk pregnancy supervision*
  NPR, ICD-10 (D): Z35.x

- *Maternal diagnosis of venous complications or hemorrhoids*
  NPR, ICD-10 (D): O22.x

- *Maternal diagnosis of chronic hypertension*
  NPR, ICD-10 (D): I10.x

- *Maternal diagnosis of gestational diabetes*
  NPR, ICD-10 (D): O24.4x

- *Maternal diagnosis of other (non-gestational) diabetes*
  NPR, ICD-10 (D): O24.0x, O24.1x, O24.3x, O24.5x, O24.9x

- *Maternal diagnosis of other illness complicating birth**
  MBR, individual-level value ascertained directly

  NPR, ICD-10 (D): O98.x

- *Maternal diagnosis of other pregnancy complications**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O26.6x, O35.9x, O36.0x, O36.1x

- *Maternal admission to general practitioner during pregnancy*
  MBR, individual-level value ascertained directly

- *Maternal admission to midwife during pregnancy*
  MBR, individual-level value ascertained directly

- *Maternal admission to obstetrician-gynecologists or other specialists during pregnancy**
  MBR, individual-level value ascertained directly
  NPR

Hospital	Department(s)
1301	52x
1330	16x
1502	04x
1516	04x
2000	25x
3800	B9x, J3x, P6x, S2x, W9x
4202	07x
5001	04x
5002	11x
5003	07x
5501	04x
6007	04x
6200	33x
6650	29x
7005	04x
8001	08x
8003	04x
7603	10x
9001	02x

Covariates – Group 4 – characteristics at birth event

- *Child place of birth (home vs hospital)*
  MBR, individual-level value ascertained directly

- *Child born in a university-affiliated hospital*
  MBR, individual-level value ascertained directly

- *Premature rupture of fetal membranes**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O42.x

- *Preeclampsia*
  NPR, ICD-10 (D): O14.x

- *Breech or other abnormal presentation**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O64.1x

- *Placenta previa**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O44.x

- *Gestational age at birth*
  MBR, individual-level value ascertained directly

- *Epidural analgesia during labor**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (B): ABZ00x

- *Any surgical induction during labor**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (K): MACx

- *Operative vaginal delivery (vacuum or forceps extraction)**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (K): MAEx, MAFx

- *Amnioinfusion during labor**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (K): MAC20x, MAC30x

- *Maternal birth injury**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O70.0x, O70.1x, O70.2x, O70.3x

- *Episiotomy**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (K): TMD00x

- *Surgery to repair maternal birth injury**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (K): MBC.x

- *Cesarean delivery for this birth**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O82.x
  NPR, ICD-10 (K): MCA10x

- *Cesarean delivery for this birth, upon maternal request**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (K): ZYM00x

- *Planned cesarean delivery for this birth**

MBR, individual-level value ascertained directly
NPR, ICD-10 (D): O82.0x
NPR, ICD-10 (K): MCA10Bx

- *Emergency cesarean delivery for this birth**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O82.1x
  NPR, ICD-10 (K): MCA10Ax, MCA10Cx, MCA10Dx, MCA10Ex

- *Post-partum hemorrhage or bleeding**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O72.x

- *Fixed placenta or fetal membranes**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O73.x

- *Navel cord prolapse*
  MBR, individual-level value ascertained directly

- *Test of scalp pH to assess fetal asphyxia**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (B): MBA03x

- *Fetal asphyxia**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): O36.3x, O68.x

- *Apgar score 5 minutes post-partum*
  MBR, individual-level value ascertained directly

- *Birth weight*
  MBR, individual-level value ascertained directly

- *Birth length*
  MBR, individual-level value ascertained directly

- *Placental weight*
  MBR, individual-level value ascertained directly

<u>Covariates – Group 5 – characteristics of perinatal period</u>

- *Mother's length of hospital stay for birth*
  MBR, individual-level value ascertained directly

- *Child's length of hospital stay after birth*

MBR, individual-level value ascertained directly

- *Child's length of hospital stay in neonatal intensive care unit (NICU)*
  MBR, individual-level value ascertained directly

- *Child, continuous positive airway pressure administered in NICU**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (B): GFC32x

- *Child, respiratory aid in NICU**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (B): GDA0x

- *Child diagnosis of sepsis during first month of life**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): P36.x

- *Child diagnosis of conditions originating in the perinatal period*
  NPR, ICD-10 (D): P0x, P1x, P2x, P5x, P6x, P7x, P8x, P9x

- *Child diagnosis of congenital malformation during first year of life**
  MBR, individual-level value ascertained directly
  NPR, ICD-10 (D): Qx

Description of geographic covariate data

Until 31 December 2006, Denmark was divided into 270 districts. On 1 January 2007, its government consolidated those districts into 98 municipalities, and allocated each municipality to one of five regions. To compare geographic data over the entire study period, we harmonized data according to the 98-municipality data structure, and used national census data taken on 1 January 2012 (available from Statistics Denmark, http://www.StatBank.dk/bev22) to assign each municipality a value for population density (number of residents per square kilometer).

We assigned geographic location for municipality and region for the child's birth (using data from the MBR) and their residence following birth (using the CRS). Home births were classified by the mother's residence (using the CRS) on the child's date of birth. For hospital births, we used a historical list of hospitals obtained from the Health Care Classification System[153–155] (http://www.medinfo.dk/sks/brows.php, ftp://filer.sst.dk/filer/sks/data/skscomplete/) and conducted online searches to identify and confirm each hospital's municipality and region, and classified each hospital as university-affiliated or not. There were five hospital codes in the historical list that pertained to groups of neighbouring hospitals, and we assigned each of those codes to the most densely populated municipality included in that code's coverage area. For example, Fredericia Hospital and Kolding Hospital were <30 km apart and were grouped together as #6007; because Fredericia Municipality's population density was approximately twice that of Kolding Municipality, we assigned #6007 to Fredericia Municipality.

APPENDIX E: SUPPLEMENTAL METHODOLOGICAL DETAIL, PROPENSITY SCORE ANALYSIS

Missing data

All eight covariates with missing data were continuous or discrete-numeric: parity (1.5% of values missing), maternal pregravid weight (5.1%) and BMI (5.9%), gestational age at birth (0.3%), Apgar score 5 minutes post-partum (0.9%), birth weight (0.6%), birth length (1.4%), and placental weight (2.8%).

Bronchitis, bronchiolitis, and respiratory syncytial virus diagnosis during the first year of life

Unmasking bias or detection signal bias[114,117,118] can arise when an exposure induces symptoms that lead to a search for disease and potential diagnosis of the outcome. This is similar to protopathic bias, which can arise when underlying preclinical symptoms of the outcome of interest affect the treatment of interest. The two biases are distinct from one another, however, because unmasking bias occurs based on the extent of searching to detect and diagnose an outcome related to symptoms which were observed *following* treatment (rather than *before* treatment as is the case with protopathic bias). In the context of antibiotics and asthma, this could occur if a patient infected with respiratory syncytial virus (RSV) were mistakenly treated with antibiotics for a suspected bacterial infection. If the viral infection proceeded to cause bronchitis, bronchiolitis or wheeze,[41] then the antibiotic would likely be recognized as ineffective and the rule-out diagnosis of bacterial infection would likely be deemed untrue or insufficient. Regardless, the healthcare claims records would still reflect the occurrence of a redeemed antibiotic prescription. As wheeze symptoms may be persistent,[41] the practitioner could subsequently prescribe treatment related to asthma; in such a scenario, the record of an antibiotic prescription would be an artefact of the practitioner's differential diagnosis method rather than as

a cause of asthma. Although diagnosis of wheeze during infancy was exceedingly rare in this population (4 children out of 542,237), controlling for diagnosis of bronchitis, bronchiolitis, and other outcomes related to respiratory syncytial virus during infancy was carried out reduce potential unmasking bias and protopathic bias.

Residual confounding in dose-response estimation

In the portion of the dose-response analysis that compares one redeemed antibiotic prescription versus none, there is more potential for residual confounding (*e.g.*, by indication) because the unexposed group may have pronounced (unmeasured) differences from the exposed which bias data interpretation. For the other four discrete dose-response comparisons between subgroups of the exposed, such confounding is likely minimized.

Heterogeneity of effects by age

In the PS-based analyses to assess for heterogeneity of risk differences by age at first redeemed antibiotic prescription, for each month of age $m=\{0, 1, 2, ..., 12\}$, we enumerated a cohort comprising infants who redeemed their first antibiotic prescription that week (the exposed group) or remained naïve to antibiotic prescription between birth and month m (unexposed). For each month separately, we used SMR weighting to balance covariate distributions across exposure groups and estimated the ATT risk difference for antibiotic exposure. Across the series of analyses by month of age, infants classified as exposed in month m did not contribute to risk difference estimates from month $m+1$ through 12.

The apparent increase in standardized risk differences across the first 3-4 months of life may describe a weaker effect of very early antibiotic exposure on treated airway disease;

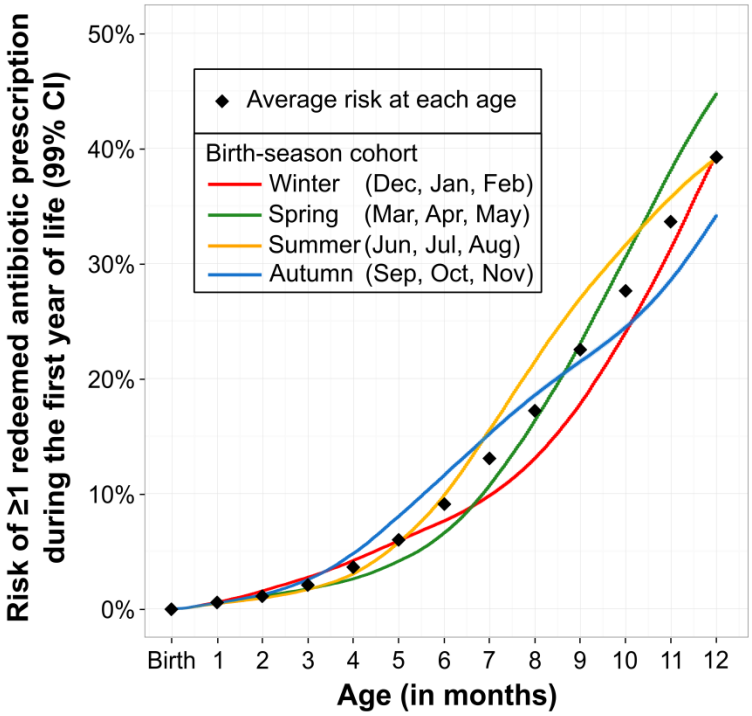
however, it may reveal exposure misclassification or unmeasured confounding in early life, despite our having restricted to children not admitted to neonatal intensive care. The optimal conditioning set of covariates is likely different for each age-specific exposure contrast; given that 91% of infants' first redeemed antibiotic prescription occur between 4-12 months of age, characteristics measured during infancy may enhance confounding control for contrasts later in infancy.

APPENDIX F: SUPPLEMENTAL METHODOLOGICAL DETAIL, INSTRUMENTAL VARIABLE ANALYSES

Defining the birth-season instrumental variable: the problem

Figure F.1 shows the cumulative incidence functions for antibiotic exposure by age through the first year of life, stratified by birth-season. It illustrates the need to consider more than the strength of the instrument-exposure relation when selecting the optimal instrument.

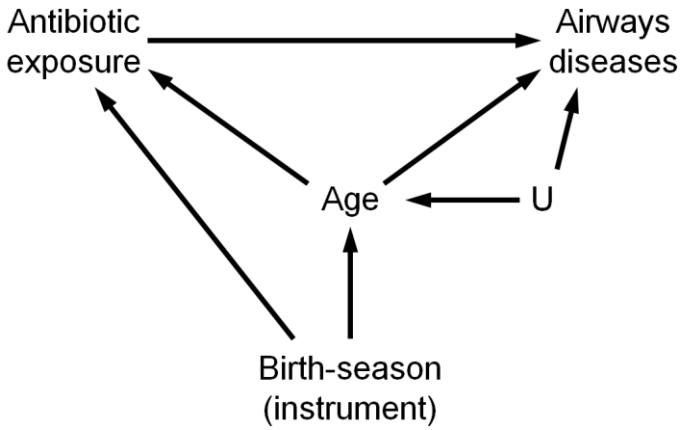
Figure F.1. Risk function for at least one redeemed antibiotic prescription, by age (in months) through the first year of life, stratified by season of birth, Denmark 2004-2012.



Season of birth is associated with incidence of antibiotic exposure; however, in addition to being associated with a pointwise difference in risk at 12 months for example, the relation between season of birth and risk of antibiotic exposure varies with increasing age. This variation is shown in Figure F.1 by the interweaving risk functions for each season of birth, leading to inconsistent differences between seasons depending on the age at comparison. Given that age[36,168] at first antibiotic exposure may be an important modifier of the association between

antibiotics and treated airway diseases, the effect of the birth-season instrument on age at first exposure could cause potential violation of the 'exclusion restriction' assumption for IV analysis. This is the condition that requires the instrument may only be associated with the outcome through the exposure; a violation would arise from the causal pathway: birth-season → age-at-exposure → treated-airway-diseases. Additionally, violation of the 'random assignment' assumption could arise in the presence of an association between birth-season and age-at-exposure if any non-causal (i.e., biasing) pathways exist between age-at-exposure and treated-airway-diseases, which is likely. The directed acyclic graph in Figure F.2 shows the potential violations that arise when there is an association between the birth-season instrument and age.

Figure F.2. A simplified directed acyclic graph for the birth-season instrument context.



In addition to increasing the potential for violation of IV assumptions, an association between the birth-season instrument and age at first antibiotic exposure would hinder meaningful interpretation of risk difference estimates from the IV analysis. Consider a binary instrument that may at first appear (from Figure F.1) to be the best available option because its association with risk of antibiotic exposure during infancy is the strongest at 12 months of age. This instrument would compare spring (i.e., March, April, May) with autumn (i.e., September, October,

November) births, and would be associated with a 12-month risk difference for antibiotic exposure, 44.8–34.3 = 10.5%. However, the exposure difference between spring and autumn births was inconsistent in sign and disproportionate in magnitude across ages (Table F.1).

Table F.1. Antibiotic exposure by age (in months), comparing spring and autumn births, Denmark 2004-2012.

	Percent exposed (%)		
Age (months)	Spring	Autumn	Difference (%)
3	2	3	-0.8
6	7	12	-5.0
9	23	22	1.6
12	45	34	10.5

If the instrument-exposure association is inconsistent with age (or during any time period when the effect of exposure on outcome occurrence is in question), interpretation may become complicated. For the purposes of illustration, let us assume the 'reduced-form' or 'intention-to-treat' IV estimate for the risk difference at 5 years of age was 2%, using the spring/autumn instrument. (For every 100 infants who redeemed an antibiotic prescription during their first year of life, 2 more would have been treated for airway diseases by 5 years of age, compared to every 100 infants who did not redeem an antibiotic prescription.) Using a simple sign test, our observation that spring births had 10.5% higher antibiotic exposure at 12 months would suggest that antibiotic exposure increased the risk for treated airway diseases (LATE $\approx 0.02 \div 0.105$).

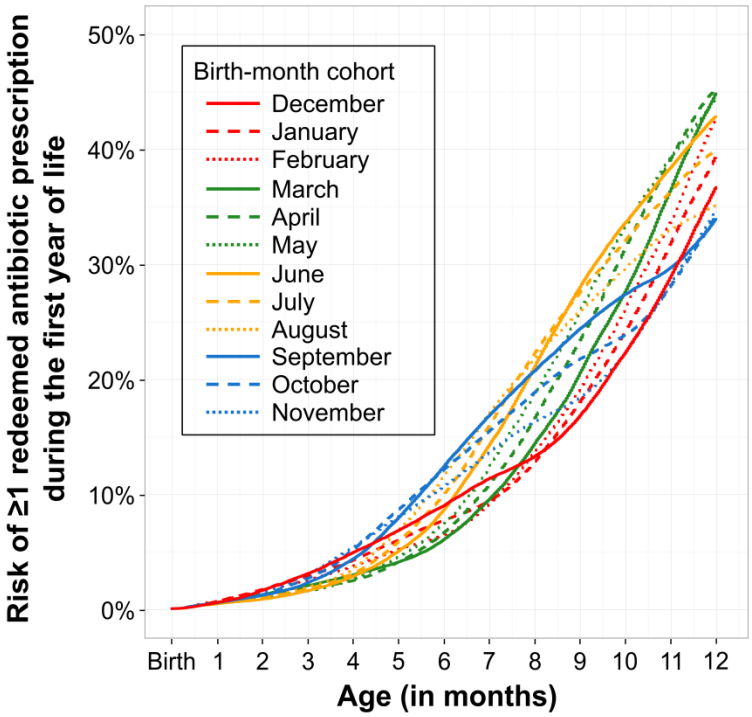
On the other hand, if we only had data on – or only thought to observe – antibiotic exposure data at 6 months of age, our interpretation of the estimate would contradict the above. Recall from Table F.1 that spring birth was associated with lower antibiotic exposure at 6 months compared to autumn births (-5%). Observing the instrument-outcome association were positive (2%), we would deduce a negative association between antibiotic exposure and treated airway

diseases (LATE ≈ 0.02 ÷ (-0.05)). Especially given the hypothesis that younger infants have a less stable microbial structure than older infants[204] and may therefore be more susceptible to adverse effects from disruptions of the microflora – it may be implausible to conclude that antibiotic exposure during the first six months decreased risk of treated airway diseases, but exposure during months 6-12 led to increased risk of treated airway diseases.

Defining the birth-season instrumental variable: approach

If a birth-season instrument could be identified such that the association between birth-season and age were minimized (to the extent that it would be plausible to remove the corresponding arrow in Figure F.2), then violation of IV assumptions would be less probable, and interpretation more straightforward. To optimize the instrument, we selected birth-month contrasts that exhibited the largest difference[60] in antibiotic exposure without being associated with age at first redeemed antibiotic prescription, thus reducing potential violation of assumptions, since age[36,168] at first antibiotic exposure may modify the association between antibiotics and treated airway diseases. We therefore examined each birth-month's cumulative incidence function for antibiotic exposure by age during the first year of life, in search of two contrastable season-based time periods with functions that did not cross and have conflicting difference measures across the age continuum. Figure F.3 (on following page) is similar to Figure F.1, but displays exposure functions stratified by the more granular classification of birth-month, showing the continuous nature of the interaction between birth-season and age at first exposure.

Figure F.3. Risk function for at least one redeemed antibiotic prescription, by age (in months) through the first year of life, stratified by month of birth, Denmark 2004-2012.



We sought birth-season instruments that would lead to as little violation of assumptions 2 and 3 as discussed above, while maintaining as much strength as possible for the instrument-exposure association. For exposure at 12 months, we observed that the optimal instrument compared children born in March and April (index level of the instrument) with children born in December and January (referent). Grouping months together in each arm of the instrument led to the most optimal weak association between the instrument and age at first exposure, thus reducing potential for violating assumptions 2 and 3. As shown in Figure F.4 and Table F.2, antibiotic exposure in the first 6 months was similar for both levels of the instrument. From 7 months of age onward, the instrument was associated with differences in exposure, resulting in a cumulative difference of 7.0%, among the largest of all candidate birth-month comparisons.

Figure F.4. 12-month risk function for at least one redeemed antibiotic prescription by age (in months), stratified by birth-season instrument level, Denmark 2004-2012.

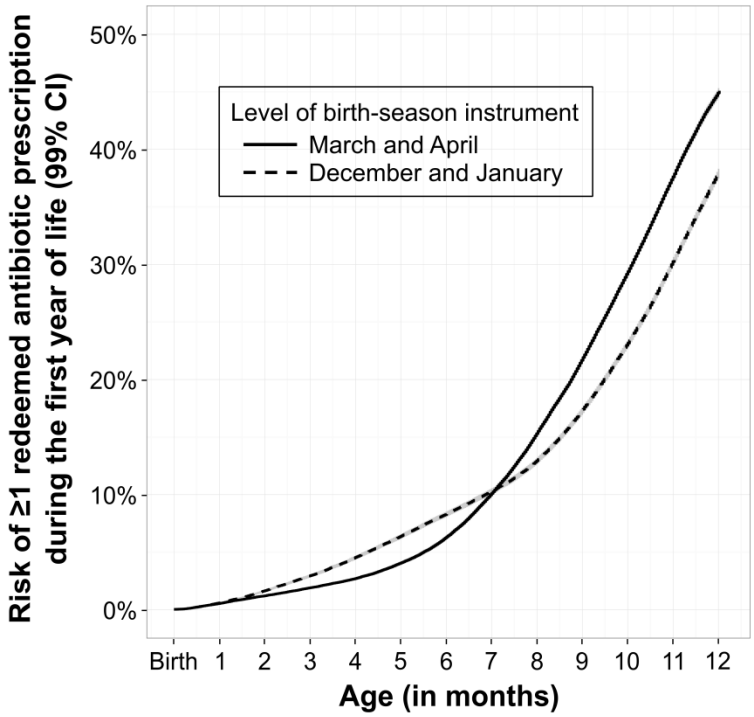


Table F.2. Antibiotic exposure by age (in months), comparing March/April and December/January births, Denmark 2004-2012.

	Percent exposed (%)		
Age (months)	Mar/Apr	Dec/Jan	Difference (%)
3	2	3	-1.1
6	6	8	-2.0
9	22	17	4.5
12	45	38	7.0

For antibiotic exposure at 9 months, we observed that the optimal instrument compared children born in July and August (index) with children born in December and January (referent). Exposure was similar across levels of the instrument until 5 months of age; from 5 to 9 months, the instrument was associated with differences in exposure, resulting in a cumulative difference of 9.3% (Figure F.5, Table F.3).

Figure F.5. 9-month risk function for at least one redeemed antibiotic prescription by age (in months), stratified by birth-season instrument level, Denmark 2004-2012.

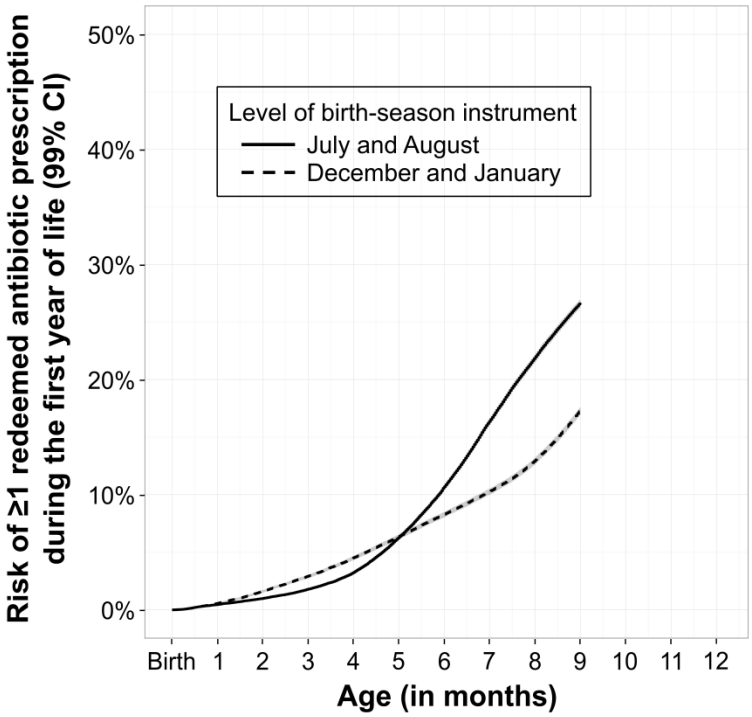
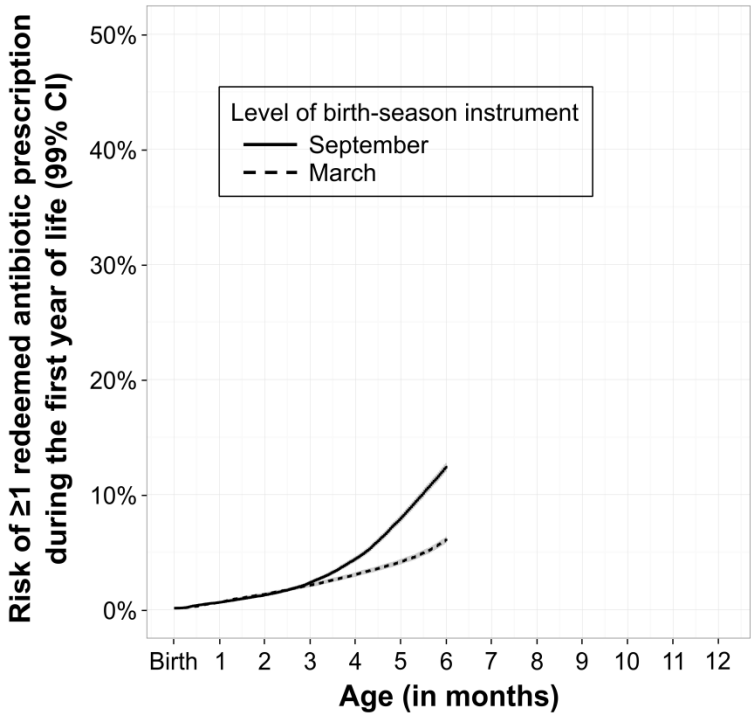


Table F.3. Antibiotic exposure by age (in months), comparing July/August and December/January births, Denmark 2004-2012.

	Percent exposed (%)		
Age (months)	Jul/Aug	Dec/Jan	Difference (%)
3	2	3	-1.1
6	11	8	2.4
9	27	17	9.3

For antibiotic exposure at 6 months, we observed that the optimal instrument compared children born in September (index) with children born in March (referent). Exposure was similar until 3 months of age; from 3 to 6 months, the instrument was associated with differences in exposure, resulting in a cumulative difference of 6.3% (Figure F.6).

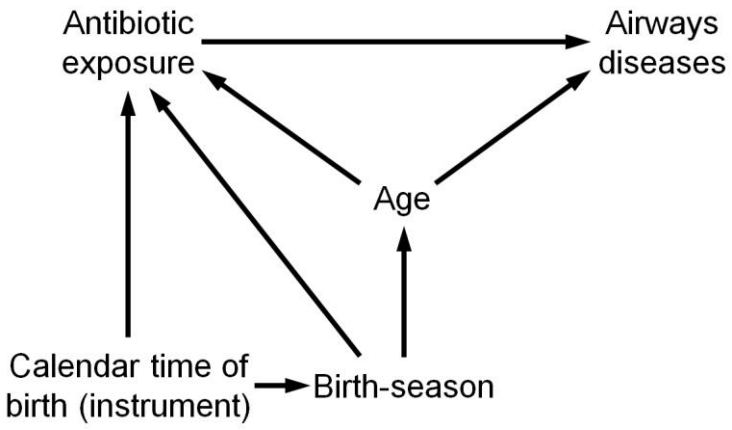
Figure F.6. 6-month risk function for at least one redeemed antibiotic prescription by age (in months), stratified by birth-season instrument level, Denmark 2004-2012.



Defining the calendar-time instrumental variable

The directed acyclic graph in Figure F.7 (on following page) is an extension of Figure F.2, in that it shows how the calendar-time instrument relates to other variables in this setting. We observed that calendar time is only associated with age at first antibiotic exposure through the intermediate of birth-season. The concern regarding the relation between birth-season and age applies in the context of the calendar-time IV analysis if the calendar-time instrument were associated with birth-season, since it would open both causal and non-causal paths between instrument and outcome in addition to the (intended) causal path from instrument through exposure to outcome.

Figure F.7. A simplified directed acyclic graph for the calendar-time instrument context.



To minimize potential bias induced by the relation between calendar time and season of birth, we based our instrument on a comparison of birth cohorts that shared the same season of birth. Thus, we implemented restriction to realize conditional independence between calendar time and season of birth, as shown in Figure F.8.

Figure F.8. A simplified directed acyclic graph for the context of the calendar-time instrument restricted to similar seasons of birth.

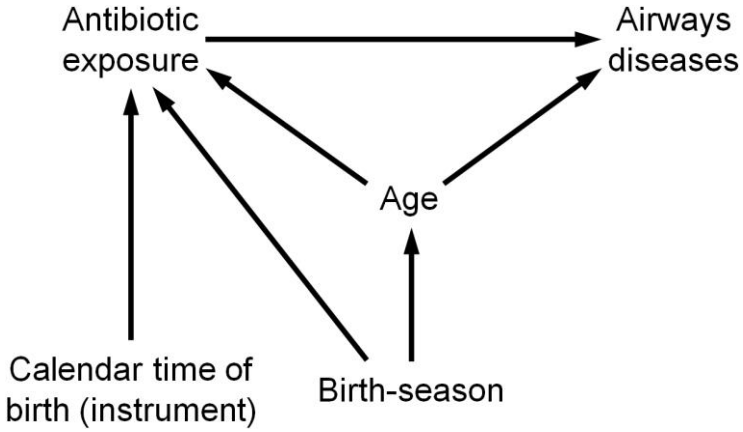
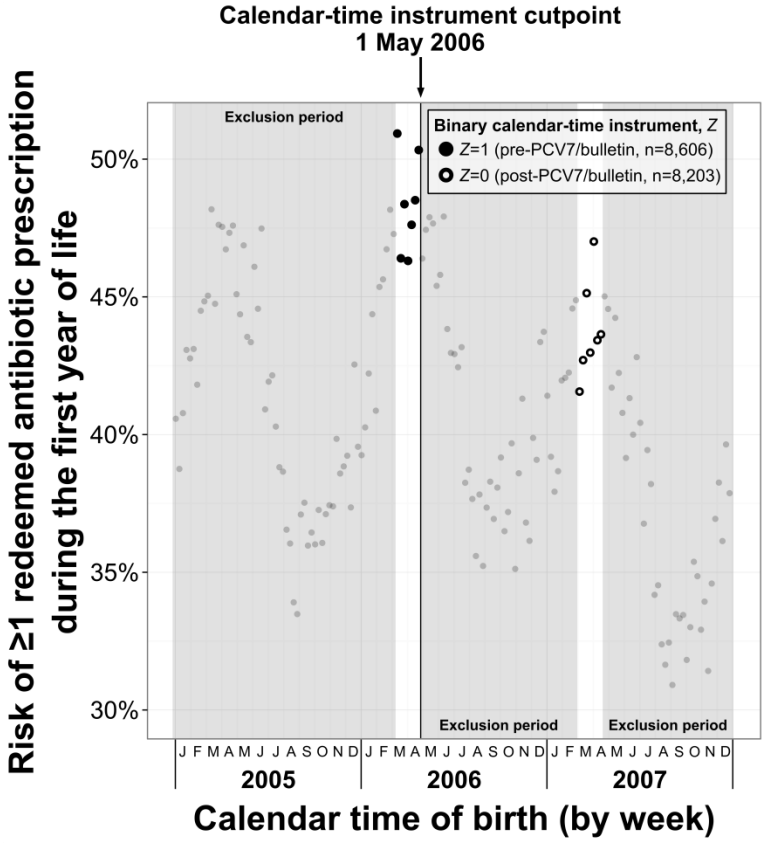


Figure F.9 shows exposure data over time that contributed to the specification of the calendar-time instrument.

Figure F.9. Index and referent levels of the calendar-time instrument, shown as groups of birth-week-specific risks of at least one redeemed antibiotic prescription during the first year of life, Denmark, 2005-2007.



Using 1 May 2006 as the cutpoint for the pre- versus post- definition of the calendar-time instrument, we defined the index level of the instrument as births occurring from 12 March 2006 through 29 April 2006 (7 weeks). Based on the birth-season restriction and the restriction of the time between instrument levels to one year, we defined the referent level of the instrument as births occurring one year later. To strengthen the instrument while maintaining near complete overlap of seasons, we used 5 March 2007 through 22 April 2007 as the referent. We excluded all other birth-week cohorts from the calendar-time IV analysis.

In a sensitivity analysis, we strengthened the association between instrument and exposure by trimming birth-week cohorts that weakened the association. For both levels, we trimmed 19-25 of March and 2-8 April; these were the two lowest internal risk values in the

index level of the instrument, and the two highest the following year for the referent level. Because these trimmed birth-week cohorts were internal to the time windows for each level, and because the differences between their risk estimates and the surrounding weeks can be considered due to random error, we used the sensitivity analysis to explore the potential impact of a stronger instrument on inference.

APPENDIX G: TABLES DESCRIBING SELECTED CHARACTERISTICS RELATED TO THE 'ANY-VERSUS-NONE,' 'ANY-AMOXICILLIN,' AND 'FIRST-ANTIBIOTIC' EXPOSURE CONTRASTS.

Table G.1. Extended table describing selected characteristics of infants born in Denmark during 2004-2012, by level of antibiotic exposure during the first year of life ('any-versus-none') in observed data and stabilized standardized morbidity ratio (SMR) weighted data; N=541,336.

	Observed data			Weighted data	
	Exposed n = 214,256 %	**Unexposed n = 327,080 %**	**SMD**	**Unexposed mw = 1.005 %**	**SMD**
Male sex	55.8	48.2	0.15	55.9	0.00
Birth order (mother's live births only)			0.17		0.01
First-born	39.4	47.7		39.1	
Second	41.1	34.8		41.4	
Third	14.8	13.4		14.9	
Fourth	3.5	3.0		3.4	
Fifth or higher	1.3	1.0		1.2	
Year of birth			0.07		0.01
2004-2006	36.4	33.2		36.8	
2007-2009	33.8	34.2		33.7	
2010-2012	29.8	32.5		29.5	
Season of birth			0.16		0.01
Winter (Dec, Jan, Feb)	23.5	23.3		23.8	
Spring (Mar, Apr, May)	28.5	22.8		28.4	
Summer (Jun, Jul, Aug)	26.6	26.9		26.3	
Autumn (Sep, Oct, Nov)	21.4	27.0		21.4	
Maternal age at birth, years (median, IQR)	(30, 27-34)	(31, 28-34)	-0.10	(30, 27-34)	0.00
Region of residence at birth			0.18		0.00
Capital Region	32.9	33.5		32.7	
Zealand Region	14.9	11.6		14.9	
Southern Region	22.9	18.9		22.9	
Central Region	19.8	26.0		19.8	
North Region	9.6	10.1		9.7	
Population density of municipality of residence at birth, residents per km^2 (median, IQR)	(161, 85-744)	(177, 87-794)	-0.08	(161, 85-744)	0.00
Age at first redeemed antibiotic prescription			--		--
≤6 months	22.6	0.0		0.0	
7-9 months	34.2	0.0		0.0	
10-12 months	43.2	0.0		0.0	
Mother, no. antibiotic prescriptions during pregnancy*			0.16		0.00
0	63.2	70.3		63.3	
1	22.2	19.5		22.2	
≥2	14.6	10.3		14.5	
Mother, rate of antibiotic prescription†‡			0.28		0.00
0	15.8	22.3		15.8	

>0-0.5	34.8	40.8		34.9	
>0.5-1	27.2	23.4		27.3	
>1	22.2	13.5		22.0	
Father, rate of antibiotic prescription†‡			0.17		0.00
0	34.7	41.0		34.7	
>0-0.5	44.2	43.5		44.2	
>0.5-1	15.2	11.7		15.1	
>1	6.0	3.9		6.0	
Older siblings, combined rate of antibiotic prescription†‡			0.39		0.01
0	43.3	54.3		42.9	
>0-1	20.7	25.7		20.8	
>1-2	18.0	12.6		18.2	
>2	18.0	7.3		18.1	
Mother, history of any obstructive airway disease†	16.1	12.9	0.09	16.0	0.00
Father, history of any obstructive airway disease†	12.0	10.8	0.04	12.0	0.00
Any older sibling, history of any obstructive airway disease†	22.3	15.2	0.18	22.5	-0.01
Diagnosis of otitis media†	2.4	0.2	0.19	2.2	0.00
Diagnosis of bronchitis, bronchiolitis, RSV pneumonia†	5.1	2.0	0.16	5.1	-0.01
Infant, no. admissions to pediatrician†			0.27		0.01
0	69.1	80.1		69.0	
1	16.6	12.7		16.7	
≥2	14.3	7.2		14.2	
Maternal smoking during pregnancy*			0.09		0.01
Did not smoke	84.5	87.5		84.4	
Smoking, amount unknown	0.3	0.3		0.3	
Stopped smoking in first trimester	2.1	2.0		2.1	
Stopped smoking after first trimester	0.5	0.4		0.5	
Smoking, ≤5 cigarettes/day	3.8	3.1		3.8	
Smoking, 6-10 cigarettes/day	4.8	3.7		4.9	
Smoking, 11-20 cigarettes/day	3.5	2.6		3.5	
Smoking, >20 cigarettes/day	0.5	0.4		0.5	
Mother, no. visits to GP during pregnancy*			0.05		0.00
0	14.5	16.1		14.6	
1-2	17.9	18.0		17.9	
3-4	65.5	64.2		65.4	
≥5	2.0	1.7		2.0	
Mother, no. visits to midwife during pregnancy*			0.05		0.01
0	7.1	8.1		7.0	
1-2	3.4	3.7		3.5	
3-4	29.5	30.4		29.6	
≥5	59.9	57.9		59.8	
Mother, no. visits to ob-gyn during pregnancy*			0.10		0.00
0	21.4	24.4		21.3	
1-2	48.2	48.8		48.2	

3-4	20.5	18.8		20.6	
≥5	10.0	8.0		9.9	
Gestational age at birth, weeks			0.04		0.01
<37	6.2	6.6		6.4	
37-39.9	43.3	41.4		43.3	
≥40	50.5	52.0		50.3	
Operative vaginal delivery	7.4	8.5	-0.04	7.4	0.00
Cesarean delivery for this birth	22.7	21.7	0.03	22.8	0.00
Cesarean delivery upon maternal request	3.2	2.6	0.04	3.2	-0.01
Emergency cesarean delivery	12.8	12.8	0.00	12.8	0.00

SMD = standardized mean difference (mean difference divided by pooled standard error)
mw = mean weight
IQR = interquartile range
RSV = respiratory syncytial virus
GP = general practitioner
ob-gyn = obstetrician-gynecology specialist
* Ascertained during the 245 days preceding the child's date of birth.
† Ascertained until the child's first birthday.
‡ Rate = number of prescriptions ÷ person-years of follow-up until the child's first birthday; extreme rates of medication use were imputed with the 99.9th percentile value from their rate-specific distribution.

Table G.2. Selected characteristics of infants born in Denmark during 2004-2012, by level of antibiotic exposure during the first year of life comparing children with at least one redeemed amoxicillin prescription to children with at least one penicillin V prescription but none for amoxicillin (*i.e.*, 'any-amoxicillin') in observed data and inverse-probability-of-treatment (IPT) weighted data; N=202,576.

	Observed data			Weighted data		
	Amoxicillin n = 139,970 %	**Referent n = 62,606 %**	**SMD**	**Amoxicillin mw = 1.000 %**	**Referent mw = 0.999 %**	**SMD**
Male sex	56.3	55.1	0.02	55.9	55.7	0.00
Birth order (mother's live births only)			0.03			0.00
First-born	38.9	40.4		39.5	39.7	
Second	41.6	40.0		41.0	40.9	
Third	14.7	14.9		14.8	14.7	
Fourth	3.5	3.5		3.5	3.5	
Fifth or higher	1.3	1.3		1.3	1.2	
Year of birth			0.07			0.01
2004-2006	35.3	38.6		36.2	36.1	
2007-2009	34.7	32.3		34.1	34.3	
2010-2012	29.9	29.0		29.7	29.6	
Season of birth			0.03			0.01
Winter (Dec, Jan, Feb)	23.4	23.7		23.4	23.7	
Spring (Mar, Apr, May)	28.9	27.9		28.6	28.5	
Summer (Jun, Jul, Aug)	26.7	26.6		26.7	26.4	
Autumn (Sep, Oct, Nov)	21.0	21.9		21.3	21.4	
Maternal age at birth, years (median, IQR)	(30, 27-34)	(30, 27-34)	-0.02	(30, 27-34)	(30, 27-34)	0.00
Region of residence at birth			0.27			0.01
Capital Region	32.9	32.6		32.8	32.9	
Zealand Region	17.2	10.7		15.2	15.1	
Southern Region	24.1	20.7		22.9	22.8	
Central Region	16.9	25.2		19.5	19.5	
North Region	8.9	10.9		9.6	9.7	
Population density of municipality of residence at birth, residents per km^2 (median, IQR)	(161, 87-794)	(153, 81-672)	0.10	(161, 85-744)	(161, 84-749)	0.00
Age at first redeemed antibiotic prescription			0.03			0.01
≤6 months	23.6	17.8		21.8	21.1	
7-9 months	35.8	31.9		34.5	35.0	
10-12 months	40.5	50.3		43.7	43.9	
Mother, no. antibiotic prescriptions during pregnancy*			0.08			0.00
0	62.1	65.3		63.1	63.0	
1	22.6	21.6		22.3	22.3	
≥2	15.4	13.1		14.7	14.7	
Mother, rate of antibiotic prescription†‡			0.13			0.00
0	14.8	17.8		15.7	15.7	
>0-0.5	33.9	36.5		34.8	34.8	
>0.5-1	27.7	26.2		27.3	27.3	

>1	23.6	19.5		22.3	22.2	
Father, rate of antibiotic prescription†‡			0.08			0.00
0	33.6	37.1		34.6	34.7	
>0-0.5	44.6	43.4		44.2	44.1	
>0.5-1	15.6	14.1		15.2	15.2	
>1	6.3	5.4		6.0	6.0	
Older siblings, combined rate of antibiotic prescription†‡			0.17			0.01
0	42.5	44.7		43.3	43.5	
>0-1	19.3	23.4		20.6	20.6	
>1-2	18.2	17.7		18.0	18.0	
>2	20.0	14.2		18.1	17.9	
Mother, history of any obstructive airway disease†	16.5	15.0	0.04	16.0	15.9	0.00
Father, history of any obstructive airway disease†	12.3	11.6	0.02	12.1	12.1	0.00
Any older sibling, history of any obstructive airway disease†	23.0	21.0	0.05	22.3	22.3	0.00
Diagnosis of otitis media†	3.0	1.3	0.12	2.4	2.3	0.00
Diagnosis of bronchitis, bronchiolitis, RSV pneumonia†	5.3	4.4	0.04	5.0	5.0	0.00
Infant, no. admissions to pediatrician†			0.12			0.00
0	67.8	72.7		69.4	69.4	
1	17.0	15.9		16.6	16.7	
≥2	15.2	11.4		14.0	13.9	
Maternal smoking during pregnancy*			0.02			0.00
Did not smoke	84.3	84.6		84.4	84.5	
Smoking, amount unknown	0.3	0.3		0.3	0.3	
Stopped smoking in first trimester	2.0	2.2		2.1	2.1	
Stopped smoking after first trimester	0.5	0.5		0.5	0.5	
Smoking, ≤5 cigarettes/day	3.9	3.8		3.8	3.8	
Smoking, 6-10 cigarettes/day	4.9	4.7		4.8	4.8	
Smoking, 11-20 cigarettes/day	3.6	3.4		3.5	3.5	
Smoking, >20 cigarettes/day	0.5	0.5		0.5	0.5	
Mother, no. visits to GP during pregnancy*			0.07			0.00
0	13.7	16.1		14.6	14.7	
1-2	18.2	17.1		17.9	17.9	
3-4	66.0	64.7		65.5	65.4	
≥5	2.0	2.2		2.0	2.0	
Mother, no. visits to midwife during pregnancy*			0.05			0.00
0	6.7	7.7		7.1	7.1	
1-2	3.4	3.4		3.4	3.4	
3-4	29.2	30.4		29.6	29.6	
≥5	60.7	58.5		60.0	59.8	
Mother, no. visits to ob-gyn during pregnancy*			0.08			0.00
0	20.4	23.1		21.3	21.3	
1-2	48.0	48.5		48.1	48.2	
3-4	21.1	19.4		20.6	20.5	
≥5	10.5	9.1		10.0	10.0	
Gestational age at birth, weeks			0.02			0.00

<37	6.2	6.2		6.2	6.3	
37-39.9	43.6	42.8		43.3	43.2	
≥40	50.3	51.1		50.6	50.6	
Operative vaginal delivery	7.3	7.6	-0.01	7.4	7.5	0.00
Cesarean delivery for this birth	23.0	22.0	0.02	22.7	22.7	0.00
Cesarean delivery upon maternal request	3.3	2.9	0.02	3.2	3.2	0.00
Emergency cesarean delivery	12.9	12.5	0.01	12.8	12.9	0.00

SMD = standardized mean difference (mean difference divided by pooled standard error)
mw = mean weight
IQR = interquartile range
RSV = respiratory syncytial virus
GP = general practitioner
ob-gyn = obstetrician-gynecology specialist
* Ascertained during the 245 days preceding the child's date of birth.
† Ascertained until the child's first birthday.
‡ Rate = number of prescriptions ÷ person-years of follow-up until the child's first birthday; extreme rates of medication use were imputed with the 99.9th percentile value from their rate-specific distribution.

Table G.3. Selected characteristics of infants born in Denmark during 2004-2012, by level of antibiotic exposure during the first year of life comparing children whose first redeemed antibiotic prescription was for amoxicillin versus penicillin V (*i.e.*, 'first-antibiotic') in observed data and inverse-probability-of-treatment (IPT) weighted data; N=198,207.

	Observed data			Weighted data		
	Amoxicillin n = 113,652 %	**Penicillin V n = 84,555 %**	**SMD**	**Amoxicillin mw = 0.999 %**	**Penicillin V mw = 1.002 %**	**SMD**
Male sex	55.6	56.1	-0.01	55.8	55.8	0.00
Birth order (mother's live births only)			0.01			0.00
First-born	39.6	39.4		39.6	39.6	
Second	41.1	41.1		41.0	41.0	
Third	14.6	14.9		14.7	14.7	
Fourth	3.4	3.5		3.5	3.5	
Fifth or higher	1.3	1.2		1.2	1.2	
Year of birth			0.09			0.00
2004-2006	34.3	38.6		35.9	35.8	
2007-2009	35.2	32.5		34.2	34.3	
2010-2012	30.5	28.9		29.9	29.8	
Season of birth			0.01			0.00
Winter (Dec, Jan, Feb)	23.3	23.7		23.4	23.5	
Spring (Mar, Apr, May)	28.7	28.5		28.6	28.6	
Summer (Jun, Jul, Aug)	26.6	26.7		26.7	26.6	
Autumn (Sep, Oct, Nov)	21.4	21.1		21.4	21.3	
Maternal age at birth, years (median, IQR)	(30, 27-34)	(30, 27-34)	-0.01	(30, 27-34)	(30, 27-34)	0.00
Region of residence at birth			0.25			0.00
Capital Region	32.8	32.9		32.7	32.7	
Zealand Region	18.0	11.6		15.3	15.3	
Southern Region	24.2	21.1		22.8	22.8	
Central Region	16.4	23.7		19.5	19.5	
North Region	8.7	10.7		9.6	9.6	
Population density of municipality of residence at birth, residents per km^2 (median, IQR)	(161, 87-797)	(161, 84-672)	0.12	(161, 87-744)	(161, 84-749)	0.00
Age at first redeemed antibiotic prescription			0.03			0.00
≤6 months	21.5	20.1		21.0	20.6	
7-9 months	35.0	34.5		34.6	35.1	
10-12 months	43.5	45.5		44.4	44.4	
Mother, no. antibiotic prescriptions during pregnancy*			0.04			0.00
0	62.5	64.0		63.1	63.0	
1	22.5	22.0		22.3	22.3	
≥2	15.1	14.0		14.7	14.7	
Mother, rate of antibiotic prescription†‡			0.06			0.00
0	15.0	16.8		15.7	15.7	
>0-0.5	34.5	35.4		34.9	34.9	
>0.5-1	27.7	26.6		27.3	27.3	
>1	22.8	21.2		22.2	22.2	

Father, rate of antibiotic prescription†‡			0.05			0.00
0	33.7	36.1		34.6	34.7	
>0-0.5	44.8	43.5		44.3	44.2	
>0.5-1	15.4	14.7		15.1	15.1	
>1	6.2	5.7		6.0	5.9	
Older siblings, combined rate of antibiotic prescription†‡			0.07			0.00
0	43.3	43.4		43.4	43.5	
>0-1	19.8	21.9		20.6	20.6	
>1-2	17.9	18.0		18.0	18.0	
>2	19.0	16.7		18.0	18.0	
Mother, history of any obstructive airway disease†	16.2	15.8	0.01	16.0	16.0	0.00
Father, history of any obstructive airway disease†	12.2	11.8	0.01	12.0	12.0	0.00
Any older sibling, history of any obstructive airway disease†	22.2	22.2	0.00	22.2	22.2	0.00
Diagnosis of otitis media†	2.7	2.2	0.03	2.5	2.5	0.00
Diagnosis of bronchitis, bronchiolitis, RSV pneumonia†	4.9	5.0	0.00	5.0	5.0	0.00
Infant, no. admissions to pediatrician†			0.04			0.00
0	69.0	70.5		69.6	69.5	
1	16.7	16.5		16.6	16.6	
≥2	14.3	13.0		13.8	13.8	
Maternal smoking during pregnancy*			0.01			0.00
Did not smoke	84.5	84.5		84.5	84.5	
Smoking, amount unknown	0.3	0.3		0.3	0.3	
Stopped smoking in first trimester	2.0	2.1		2.1	2.1	
Stopped smoking after first trimester	0.5	0.5		0.5	0.5	
Smoking, ≤5 cigarettes/day	3.8	3.9		3.8	3.8	
Smoking, 6-10 cigarettes/day	4.8	4.8		4.8	4.8	
Smoking, 11-20 cigarettes/day	3.5	3.4		3.5	3.5	
Smoking, >20 cigarettes/day	0.5	0.5		0.5	0.5	
Mother, no. visits to GP during pregnancy*			0.07			0.00
0	13.5	15.9		14.6	14.7	
1-2	18.4	17.0		17.9	17.9	
3-4	66.1	64.9		65.5	65.4	
≥5	2.0	2.2		2.0	2.0	
Mother, no. visits to midwife during pregnancy*			0.05			0.00
0	6.7	7.6		7.1	7.1	
1-2	3.3	3.5		3.4	3.4	
3-4	29.1	30.2		29.5	29.5	
≥5	60.9	58.8		59.9	59.9	
Mother, no. visits to ob-gyn during pregnancy*			0.06			0.00
0	20.5	22.6		21.4	21.3	
1-2	48.1	48.1		48.0	48.0	
3-4	21.1	19.7		20.6	20.6	
≥5	10.3	9.6		10.0	10.0	
Gestational age at birth, weeks			0.01			0.00
<37	6.2	6.1		6.2	6.2	

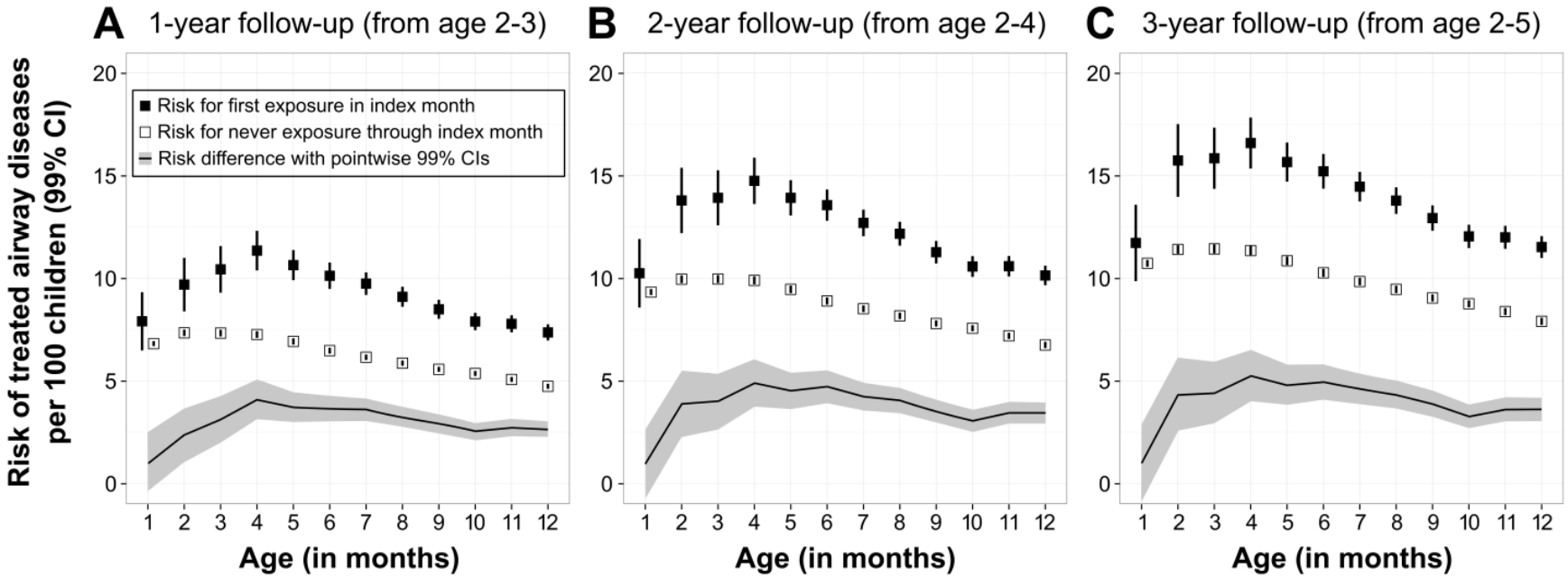
37-39.9	43.3	43.1		43.2	43.2	
≥40	50.4	50.8		50.6	50.6	
Operative vaginal delivery	7.3	7.6	-0.01	7.4	7.5	0.00
Cesarean delivery for this birth	22.9	22.4	0.01	22.7	22.7	0.00
Cesarean delivery upon maternal request	3.2	3.1	0.01	3.2	3.2	0.00
Emergency cesarean delivery	12.9	12.6	0.01	12.8	12.8	0.00

SMD = standardized mean difference (mean difference divided by pooled standard error)
mw = mean weight
IQR = interquartile range
RSV = respiratory syncytial virus
GP = general practitioner
ob-gyn = obstetrician-gynecology specialist
* Ascertained during the 245 days preceding the child's date of birth.
† Ascertained until the child's first birthday.
‡ Rate = number of prescriptions ÷ person-years of follow-up until the child's first birthday; extreme rates of medication use were imputed with the 99.9th percentile value from their rate-specific distribution.

APPENDIX H: SENSITIVITY ANALYSIS OF RISK DIFFERENCE HETEROGENEITY BY AGE AT FIRST ANTIBIOTIC EXPOSURE, OMITTING CONTROL OF COVARIATES MEASURED DURING INFANCY.

Figure H.1, below, shows higher risk differences and a more pronounced decreasing trend in the risk difference from 4-10 months of age, compared to results from the primary analysis.

Figure H.1. Risk difference heterogeneity by age at first redeemed antibiotic prescription for the relation between antibiotic exposure and risk of treated airway diseases among children born in Denmark, 2004-2012, who were never admitted to neonatal intensive care.



APPENDIX I: BIAS POTENTIAL AND IMPRECISE ESTIMATION OF THE LOCAL AVERAGE TREATMENT EFFECT IN INSTRUMENTAL VARIABLE ANALYSES

Table I.1. Summary measures of covariate imbalance between levels of the instrument in observed and weighted data based on the average standardized absolute mean difference (SAMD) and the average local average treatment effect (LATE) bias.

	Observed data		Weighted data	
Instrumental variable	**Average SAMD**	**Average LATE bias***	**Average SAMD**	**Average LATE bias***
Birth-season, 12 months†	0.02	0.24	0.001	0.02
Birth-season, 9 months‡	0.02	0.18	0.001	0.01
Birth-season, 6 months§	0.02	0.25	0.002	0.03
Calendar-time, primary‖	0.03	0.70	0.003	0.07
Calendar-time, enhanced¶	0.03	0.54	0.003	0.05

SAMD = standardized absolute mean difference (absolute value of the quotient for the mean difference divided by the pooled standard error)

LATE = local average treatment effect

* Average LATE bias is equal to the average SAMD scaled by the instrument's strength (average SAMD divided by compliance proportion)

† Instrument regarding exposure differences at 12 months, comparing children born in March and April versus December and January

‡ Instrument regarding exposure differences at 9 months, comparing children born in July and August versus December and January

§ Instrument regarding exposure differences at 6 months, comparing children born in September versus March

‖ Instrument regarding exposure differences at 12 months, comparing children born during 12 March-29 April 2006 versus 5 March-22 April 2007

¶ Instrument regarding exposure differences at 12 months, comparing children born during (12/3/2006-18/3/2006, 26/3/2006-1/4/2006, 9/4/2006-29/4/2006) versus (5/3/2007-18/3/2007, 26/3/2007-1/4/2007, 9/4/2007-22/4/2007)

APPENDIX J: RELATION BETWEEN TREATED AIRWAY DISEASES AND ASTHMA IN CHILDREN

In our study, children were defined as having treated airway diseases if they redeemed at least one prescription for at least two (of the three) classes of outcome-related drugs. We grouped these prescriptions into three drug classes: (1) inhaled β_2-adrenoreceptor agonists; (2) inhaled glucocorticoids; and (3) leukotriene antagonists.

To illustrate the relation between treated airway diseases before age 5 and the occurrence of asthma in later childhood in Denmark, we compared our study outcomes with asthma status at 7 years of age. We conducted this comparison within the subcohort of children in Denmark who had available data through their seventh birthday, and ascertained asthma status using data between their fifth and seventh birthday. Three comparison definitions were used: (1) the same criteria as treated airway diseases – at least one redeemed prescription for at least two classes of anti-asthma medications; (2) at least one discharge diagnosis code for asthma in the Danish National Registry of Patients (NPR), following a hospitalization, outpatient visit, or emergency department visit, and using the International Classification of Diseases Tenth Revision (ICD-10); (3) satisfaction of criteria in definitions 1 and 2. In database studies of childhood asthma, similar definitions to these have frequently been implemented.[5,6,79,116,151,205–208]

To compare outcome classification by age and criteria, or each comparison using the definitions of 7-year asthma status as a series of pseudo-gold standards,[209] we calculated a kappa coefficient,[210,211] sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with 99% confidence intervals (CIs).

Figure J.1 shows the relation between these outcomes at different ages in childhood. The absolute risk of being diagnosed with asthma between age 5 and 7 years was low (1.4%), but the proportion of children who received and redeemed prescriptions to treat airway diseases at those

ages was higher (4.4%). Among children diagnosed with asthma between age 5 and 7, 37% were treated for airway diseases by age 3, 53% were treated by age 4, and 65% were treated by age 5. Compared definitions of asthma based on prescriptions only or prescriptions and an observed diagnosis, sensitivity of the treated airway disease outcomes decreased by approximately 10% when asthma was defined based on an observed diagnosis only. Regarding specificity, among children who were not diagnosed with asthma between age 5 and 7, 94% were not treated by age 3, 91% were not treated by age 4, and 90% were not treated by age 5. The PPV was consistent at 30% for treated airway diseases at ages 3, 4, and 5 compared to age 7, but ranged 6-9% when the pseudo-gold standard incorporated an observed diagnosis to define asthma by age 7.

(Figure J.1 on following page.)

Figure J.1. Comparison between treated airway diseases before age 5 and three classifications of asthma at age 7 among children born in Denmark. The pseudo-gold standard in the left column was based on births from 2004-2008 since it relied solely on prescribing data which we obtained through 2015. The right two columns were based on births from 2004-2005, since diagnosis data were not available after 2012.

Pseudo-gold standard based on redeemed prescriptions only, age 7

1-year risk	*+ at age 7*	*+ at age 7*	
+ at age 3	6439	15146	21585
- at age 3	7292	285003	292295
	13731	300149	313880

		99% CI	
Kappa	0.33	0.32	0.34
Sensitivity	0.47	0.46	0.48
Specificity	0.95	0.95	0.95
PPV	0.30	0.29	0.31
NPV	0.98	0.97	0.98

2-year risk	*+ at age 7*	*- at age 7*	
+ at age 4	8734	20276	29010
- at age 4	4997	279873	284870
	13731	300149	313880

		99% CI	
Kappa	0.37	0.36	0.38
Sensitivity	0.64	0.63	0.65
Specificity	0.93	0.93	0.93
PPV	0.30	0.29	0.31
NPV	0.98	0.98	0.98

3-year risk	*+ at age 7*	*- at age 7*	
+ at age 5	10215	22643	32858
- at age 5	3516	277506	281022
	13731	300149	313880

		99% CI	
Kappa	0.40	0.39	0.41
Sensitivity	0.74	0.73	0.75
Specificity	0.92	0.92	0.93
PPV	0.31	0.30	0.32
NPV	0.99	0.99	0.99

Pseudo-gold standard based on asthma diagnosis only, age 7

1-year risk	*+ at age 7*	*- at age 7*	
+ at age 3	676	7836	8512
- at age 3	1128	115608	116736
	1804	123444	125248

		99% CI	
Kappa	0.11	0.10	0.12
Sensitivity	0.37	0.35	0.40
Specificity	0.94	0.93	0.94
PPV	0.08	0.07	0.09
NPV	0.99	0.99	0.99

2-year risk	*+ at age 7*	*- at age 7*	
+ at age 4	949	10506	11455
- at age 4	855	112938	113793
	1804	123444	125248

		99% CI	
Kappa	0.12	0.11	0.13
Sensitivity	0.53	0.50	0.56
Specificity	0.91	0.91	0.92
PPV	0.08	0.08	0.09
NPV	0.99	0.99	0.99

3-year risk	*+ at age 7*	*- at age 7*	
+ at age 5	1166	11906	13072
- at age 5	638	111538	112176
	1804	123444	125248

		99% CI	
Kappa	0.13	0.12	0.14
Sensitivity	0.65	0.62	0.68
Specificity	0.90	0.90	0.91
PPV	0.09	0.08	0.10
NPV	0.99	0.99	0.99

Pseudo-gold standard based on prescriptions and asthma diagnosis, age 7

1-year risk	*+ at age 7*	*- at age 7*	
+ at age 3	514	7998	8512
- at age 3	625	116111	116736
	1139	124109	125248

		99% CI	
Kappa	0.09	0.08	0.10
Sensitivity	0.45	0.41	0.49
Specificity	0.94	0.93	0.94
PPV	0.06	0.05	0.07
NPV	0.99	0.99	1.00

2-year risk	*+ at age 7*	*- at age 7*	
+ at age 4	713	10742	11455
- at age 4	426	113367	113793
	1139	124109	125248

		99% CI	
Kappa	0.10	0.09	0.11
Sensitivity	0.63	0.59	0.66
Specificity	0.91	0.91	0.92
PPV	0.06	0.06	0.07
NPV	1.00	1.00	1.00

3-year risk	*+ at age 7*	*- at age 7*	
+ at age 5	857	12215	13072
- at age 5	282	111894	112176
	1139	124109	125248

		99% CI	
Kappa	0.11	0.10	0.11
Sensitivity	0.75	0.72	0.79
Specificity	0.90	0.90	0.90
PPV	0.07	0.06	0.07
NPV	1.00	1.00	1.00

APPENDIX K: INDIVIDUAL-LEVEL CHARACTERISTICS OF TREATED AIRWAY DISEASES OVER TIME

To examine the stability in outcome classification in more granular time periods from year to year, we assessed agreement of treated airway diseases across discrete years of age from 2 to 5. The tables below show trajectories of 'current' treated airway diseases status across distinct follow-up periods by age (in years) up to age 5. As in the primary analysis, treated airway diseases was defined as redeeming at least one prescription from at least two classes of drugs for obstructive airway diseases. To be classified with the outcome at a specific age, both redemptions had to occur within that year of age.

Table K.1. 'Current' treated airway diseases (Yes/No) in each period for children observed for one year only, through 3rd birthday (n=58,176 births occurring in 2012)

Age 2-3	Age 3-4	Age 4-5	% at age 3
No	--	--	94.4
Yes	--	--	5.6

Table K.2. 'Current' treated airway diseases (Yes/No) in each period for children observed for two years only, through 4th birthday (n=58,696 births occurring in 2011)

Age 2-3	Age 3-4	Age 4-5	% at age 3	% at age 4
No	No	--	94.4	92.4
No	Yes	--		2.0
Yes	No	--	5.6	2.9
Yes	Yes	--		2.7

Table K.3. 'Current' treated airway diseases (Yes/No) in each period for children observed for three years, through 5th birthday (n=438,466 births occurring 2004-2010)

Age 2-3	Age 3-4	Age 4-5	% at age 3	% at age 4	% at age 5
No	No	No	93.4	91.4	90.4
No	No	Yes			1.1
No	Yes	No		2.0	1.3
No	Yes	Yes			0.7
Yes	No	No	6.6	3.5	3.0
Yes	No	Yes			0.6
Yes	Yes	No		3.1	1.4
Yes	Yes	Yes			1.6

Using data from Tables K.2 and K.3, we calculated sensitivity and positive predictive value (PPV) for each comparison, and report them below. Calculations comparing age 3 versus 4 are based on children born during 2004-2011, and calculations comparing age 3 versus 4 or 3 versus 5 are based on children born during 2004-2010.

Table K.4. Sensitivity and positive predictive value for 'current' treated airway diseases outcome status, comparing age 3 versus 4, age 4 versus 5, and age 3 versus 5.

	Age 3 versus 4	Age 4 versus 5	Age 3 versus 5
Sensitivity	0.60	0.59	0.56
PPV	0.46	0.46	0.33

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