Journal of Infection (2015) 71, 458–469

BIAM British Infection Association

www.elsevierhealth.com/journals/jinf

Nasopharyngeal bacterial burden and antibiotics: Influence on inflammatory markers and disease severity in infants with respiratory syncytial virus bronchiolitis☆

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Accepted 27 June 2015
Available online 3 July 2015

☆ This work was presented in part at the European Society for Pediatric Infectious Diseases 30th Annual Meeting. Thessaloniki, Greece, May 8–12, 2012.

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http://dx.doi.org/10.1016/j.jinf.2015.06.010

KEYWORDS
Nasopharyngeal bacterial colonization;
Bronchiolitis;
RSV;
Disease severity;
Gram-negative bacteria;
Antibiotics

Summary *Objectives:* Animal studies suggest that RSV increases nasopharyngeal (NP) bacterial colonization facilitating bacterial infections. We investigated the influence of antibiotic treatment and colonization with potentially pathogenic bacteria on inflammatory markers and disease severity in RSV-infected in infants.
Methods: Healthy young infants hospitalized with RSV bronchiolitis (n = 136) and age-matched healthy controls (n = 23) were enrolled and NP samples cultured for potentially pathogenic bacteria including: Gram-positive bacteria (GPB): *Staphylococcus aureus*, *Streptococcus pneumoniae*, β-hemolytic *Streptococcus*; and Gram-negative bacteria (GNB): *Moraxella catarrhalis* and *Haemophilus influenzae*. Clinical parameters and plasma IL-8, IL-6 and TNF-α concentrations were compared according to the bacterial class and antibiotic treatment.
Results: Antibiotic treatment decreased by 10-fold NP bacterial recovery. Eighty-one percent of RSV infants who did not receive antibiotics before sample collection were colonized with pathogenic bacteria. Overall, GNB were identified in 21% of patients versus 4% of controls who were mostly colonized with GPB. Additionally, in RSV patients NP white blood cell counts ($p = 0.026$), and blood neutrophils ($p = 0.02$) were higher in those colonized with potentially pathogenic bacteria versus respiratory flora. RSV patients colonized with GNB had higher plasma IL-8 ($p = 0.01$) and IL-6 ($p < 0.01$) concentrations than controls, and required longer duration of oxygen ($p = 0.049$).
Conclusions: Infants with RSV bronchiolitis colonized with potentially pathogenic bacteria had increased numbers of mucosal and systemic inflammatory cells. Specifically, colonization with GNB was associated with higher concentrations of proinflammatory cytokines and a trend towards increased disease severity.

Introduction

Respiratory syncytial virus (RSV) lower respiratory tract infections (LRTI) represent the leading cause of hospitalization in infants worldwide.[1,2] Epidemiologic studies have identified children at high risk for severe RSV disease and mortality.[3–6] Nevertheless, the majority of infants hospitalized with RSV LRTI are previously healthy with no known risk factors.[7,8] Of those, up to 15% require pediatric intensive care unit (PICU) treatment.[9]

A broad variety of bacteria colonize the children's nasopharynx, including commensal bacteria and potential pathogens such us *Streptococcus pneumoniae*, *Staphylococcus aureus*, non-typable *Haemophilus influenzae* and *Moraxella catarrhalis*.[10–12] These potentially pathogenic bacteria usually colonize the nasopharynx without causing symptoms, however when the balance between the host and the pathogen is disrupted clinical disease may occur. Studies *in vitro* and in animal models suggest that respiratory viral infections, and specifically RSV, increase nasopharyngeal (NP) bacterial colonization promoting bacterial infections.[11,13,14] The information in infants is limited. Epidemiologic studies have shown a temporal association between RSV infections and invasive pneumococcal disease.[15–20] In addition, studies mostly performed in older children with viral-induced wheezing or pneumonia suggest that colonization with pathogenic bacteria increases disease severity.[15,21–23] The potential role of NP colonization with PPB in modifying the severity of RSV LRTI remains to be defined. Although, antibiotics are not routinely recommended for the treatment of bronchiolitis, they are commonly used, likely reflecting physician concerns of an undetected bacterial infection in young infants.[24] Whether antibiotic treatment impacts NP bacterial colonization, and whether infants with RSV LRTI receiving antibiotics represent a different subset of infants with enhanced disease severity has not been well characterized. The objectives of this study were: **1)** to determine the frequency and type of NP colonization with potentially pathogenic bacteria in healthy infants hospitalized with RSV LRTI, and **2)** to assess the impact of bacterial colonization on inflammatory cells in both the upper respiratory tract and blood; on plasma inflammatory cytokines; and on clinical disease severity after adjusting for antibiotic use.

Subjects, materials, and methods

Study design

This was a prospective, observational cohort study conducted in otherwise healthy infants hospitalized with a first episode of RSV bronchiolitis and a group of healthy age-matched controls during the 2010-11 RSV season. Patients were excluded if they were premature (gestational age < 35 weeks), had chronic medical conditions, were diagnosed with other respiratory viral infections (i.e. parainfluenza virus, human metapneumovirus), immunodeficiency, or had received systemic steroids or any immunomodulatory drugs within 2 weeks of hospitalization (Fig. 1). Monday through Friday infants hospitalized with bronchiolitis were identified using the hospital census. Those who had a confirmatory RSV test (58% by direct fluorescence antibody testing (DFA); 36% by RSV rapid antigen test and 2% by a PCR panel) or had a clinical picture compatible with bronchiolitis in the peak of the RSV season and were subsequently confirmed by RSV PCR (4%) were enrolled on days 1 to 3 of hospitalization (median 24 h) when fulfilling the study criteria. Healthy controls were enrolled in the