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Individual-level effects of antibiotics on colonizing otitis pathogens in the nasopharynx

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ABSTRACT

Background: Although there is evidence of an association between antibiotic consumption and resistant bacteria on a population level, the relationship on an individual level has been less well studied, particularly in terms of nasopharyngeal colonization. We have therefore analysed this association, using data from a closely followed cohort of children taking part in a vaccination trial.
Methods: 109 children with early onset of acute otitis media (AOM) were randomised to heptavalent pneumococcal conjugate vaccine (PCV7) or no vaccination. They were followed for three years with scheduled appointments as well as sick visits. Nasopharyngeal cultures were obtained at all visits. Antibiotic treatments were recorded, as were risk factors for AOM, including siblings, short breast-feeding and parental smoking. Data were entered into a Cox regression model, and the findings of *Streptococcus pneumoniae* and *Haemophilus influenzae* with reduced susceptibility to the penicillin group were related to the number of previous courses of antibiotics.
Results: There was evidence of an association between the amount of previously consumed betalactams and colonization with beta-lactamasenegative ampicillin-resistant (BLNAR) *H. influenzae* (RR 1.21; 95% CI 1.03–1.43; p = 0.03), and also with the most commonly prescribed drug; amoxicillin (RR 1.39; 95% CI 1.09–1.76; p = 0.01). There was no evidence for an association between antibiotic consumption and betalactamase producing *H. influenzae* or *S. pneumoniae* with reduced susceptibility to penicillin. Furthermore, there was no evidence of an association between resistant bacteria and AOM risk factors or PCV7.
Conclusion: In this subgroup of children, most of whom were given several courses of antibiotics in early childhood, there was evidence of an association between betalactam/amoxicillin consumption and nasopharyngeal colonization with BLNAR strains, bacteria that have increased in prevalence during the last 10–15 years, and that are notoriously difficult to treat with oral antibiotics.

1. Introduction

Antibiotics constitute a force that promotes horizontal transfer and selection of resistant mutants. Consequently, bacterial resistance to antibiotic drugs has been an increasing problem worldwide, ever since the introduction of the first classes of antibiotics [1,2]. On a population level, there is an association between antibiotic use and high resistance rates among upper airway pathogens [1,3], but data on the individual level are more limited. It has been suggested that a suitable way of studying individual-level effects of antibiotics on colonizing bacteria would be to measure acquisition and loss rates in a cohort where subjects are serially cultured before, during, and after antibiotic therapy [4].

Since betalactams are the first-line drugs for airway infections in most countries, any type of resistance to this group of drugs will have a clinical impact. Most important in this context are *Streptococcus pneumoniae* with decreased susceptibility to penicillin (PNSP), beta-lactamase-positive ampicillin-resistant *Haemophilus influenzae* (BLPAR Hi) and beta-lactamase-negative ampicillin-resistant *H. influenzae* (BLNAR Hi). PNSP is a growing problem worldwide, particularly in Asia, where 70–80% of pneumococci are PNSP [5,6]. In Europe, the corresponding figure is 1–36%, with lower prevalences in countries with lower antibiotic consumption [7,8]. Beta-lactamase production was the first resistance

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mechanism to be described for *H. influenzae*, and it has long been the most common, although several countries have recently described a possible decline in prevalence [9]. BLNAR strains were discovered later, and had prevalence figures below 1% in most countries for many years. However, an increase has been observed during the last two decades, most notably in Japan, where 30–60% of *H. influenzae* isolates have been BLNAR [10,11]. Europe has also been affected, but to a lesser extent (2–20%) [12]. Combinations of beta-lactamase production and changes in the penicillin-binding proteins have, in addition, been described [13]. In Sweden, a country with generally low isolation frequencies of resistant bacteria [7,14], the prevalence of BLNAR Hi remained around 3% until 2009, when it started to increase [15].

In children, acute otitis media (AOM) is the most common reason for antibiotic prescriptions [16], and 10–15% of children have recurrent AOM (rAOM) [17,18]. We performed a vaccination trial in children with early onset of AOM – a strong risk factor for rAOM - and noted a relatively high number of cultures with resistant bacteria, particularly BLNAR Hi, among the study subjects. The aim of this study was to investigate how the presence of resistant bacteria related to antibiotic consumption in this cohort.

2. Materials and methods

2.1. Study design

The study was performed at the Department of Otorhinolaryngology, Head and Neck Surgery, Lund University Hospital, Sweden. The study was conducted according to Good Clinical Practice and the principles of the Declaration of Helsinki (as amended in Somerset West, South Africa 1996), and was approved by the ethics committee at Lund University. Before approval, the ethics committee demanded that no placebo or alternative vaccination was carried out in the control group. Children were included in the study between 2003 and 2007, and PCV7 was not included in the Swedish immunisation programme until 2009. The study group consisted of two study doctors, both otorhinolaryngologists, and one study nurse.

The main question was to investigate the effect of PCV7 vaccination in children with rAOM. In order to find these patients, children with an onset of AOM before six months of age were included in the study. The diagnosis had to be confirmed by an otorhinolaryngologist. Each child was followed for three years. Visits to the study doctors were carried out every other month during the first year, at the end of the three-year period, and, in addition, whenever the parents suspected a new episode of AOM. Nasopharyngeal cultures were obtained at all visits. Half of the children were randomised to receive PCV7 and the other half received no vaccination.

2.2. Vaccination

Children were randomised as required by the ethics committee. Thus, the parents were not blinded to their child's allocation to vaccine or control group, though they were asked not to reveal this to the study personnel. The randomisation list was provided by the independent external group and was decided by lot. The list was sent in a sealed envelope to a nurse at the ENT department who administered the vaccine, but was not otherwise engaged in the study. The vaccine (Prevenar®, Wyeth Lederle, now Pfizer, NY, USA) was given to the children at intervals recommended by the manufacturer. Accordingly, children younger than 6 months at start of vaccination received three intramuscular injections with an interval of at least 1 month, whereas older children received two injections with a 1 month interval. A booster injection was given during the second year of life, resulting in three or four injections in total. This design meant that, depending on when the children had their first episode of AOM, making them eligible for the study, they started vaccination at different ages, but all children had had at least one episode of AOM before being randomised.

2.3. Definitions and treatment of AOM

AOM was defined as a bulging ear drum and opaque fluid in the middle ear in a child with symptoms of an acute infection. Difficult-to-treat otitis media was defined as continued symptoms and remaining opaque fluid in the middle ear in spite of antibiotic treatment for at least 2 days. A new episode of AOM was diagnosed if the child, after completing treatment with antibiotics and having a period with no clinical signs of AOM, had new symptoms and otomicroscopic findings as described earlier. RAOM was defined as at least three episodes during a 6-month period, or four in a year. The Swedish guidelines during the study period recommended antibiotics for AOM in all children under the age of 2 years. The drug of choice was penicillin V followed by amoxicillin in difficult-to-treat AOM or in cases of quick relapses. Otherwise, antibiotics were chosen according to earlier culture findings. At each planned visit (every other month), information about infections and antibiotic treatments since the previous visit was collected and checked against medical records. At all planned visits, parents were asked whether they had sought medical treatment outside the hospital (with GP:s, on travels etc) during the previous period.

2.4. Bacterial cultures

Cultures were analysed with regards to three types of resistant bacteria: PNSP, BLPAR Hi and BLNAR Hi. Nasopharyngeal samples were collected with the M40 Transystem (Copan Diagnostics, Corona, CA, USA) and transported to the Department of Clinical Microbiology at Malmö University Hospital during 2003–2006, and to the Department of Clinical Microbiology at Uppsala University Hospital from 2007 onwards. The samples were cultured on blood and chocolate agar plates (Becton Dickinson, Sparks, MD, USA) and were incubated for 48 h in a moist environment with 5% CO2. Bacteria were identified using standard laboratory procedures. All isolates were frozen at −70 °C. Antibiotic susceptibility testing was performed using the IsoSensitest agar (Oxoid Ltd, Basingstoke, UK). The disc diffusion method and testing of beta-lactamase production was performed as recommended by the Swedish Reference Group for Antibiotics (SRGA) [19]. Minimum inhibitory concentration (MIC) determination by Etest (AB Biodisk, Solna, Sweden) was performed when indicated and according to the manufacturer's instructions. The species-related breakpoints defined by the SRGA were used for the categorization of isolates into susceptible, indeterminate, or resistant.

2.5. Data analysis

Data were analysed according to the intention-to-treat principle, analysing the data from the children who dropped out until the day they were censored. Information about the number of days on each antibiotic, the number of previous cultures, and risk factors such as gender, siblings, breast-feeding and passive smoking was collected. Each type of resistant bacterial species (PNSP, BLPAR Hi or BLNAR Hi) was analysed separately. When a child first acquired either of these bacteria, this was classified as a failure, and the child was excluded from further analysis concerning this type of resistance. The individual's previous consumption, counted as the number of 10-day courses, of betalactams (penicillin V, amoxicillin and amoxicillin with clavulanic acid) and of amoxicillin alone, being