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Antibiotic resistance of *Streptococcus pneumoniae* in children with acute otitis media treatment failure

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ABSTRACT

Objectives: The emergence of antibiotic-resistant bacteria is a major cause of treatment failure in children with acute otitis media (AOM). This study aimed to analyze the types of bacterial strains in fluid isolated from the middle ear of children with AOM who did not respond to oral antibiotic treatment. We also determined the antibiotic resistance of the most frequently isolated bacterial strain (*Streptococcus pneumoniae*) found in these children.

Methods: This was a prospective study of 157 children with AOM aged from 6 months to 7 years admitted due to unsuccessful oral antibiotic treatment. All children underwent a myringotomy, and samples of the middle ear fluid were collected for bacteriological examination.

Results: Positive bacterial cultures were obtained in 104 patients (66.2%), with *Streptococcus pneumoniae* (39.69%), *Haemophilus influenzae* (16.03%) *Staphylococcus aureus* (16.03%), *Staphylococcus haemolyticus* (6.9%) and *Streptococcus pyogenes* (5.34%) found most frequently. The majority (65.4%) of *S. pneumoniae* strains were penicillin-intermediate-resistant or penicillin-resistant, and 67.2% strains of *S. pneumoniae* were multidrug-resistant.

Conclusions: We identified *S. pneumoniae* as the most frequently isolated pathogen from the middle ear in children with AOM treatment failure and determined that the majority of strains were antibiotic-resistant. We propose that the microbiological identification of bacterial strains and their degree of antibiotic resistance should be performed prior to therapy in order to choose the most appropriate antibiotic therapy for children with AOM treatment failure.

1. Introduction

Acute otitis media (AOM) is a common infection in children caused by bacteria and viruses [1,2]. Antibiotic treatment of AOM is usually empiric, as the causative pathogens are not routinely identified prior to therapy due to the risks associated with tympanocentesis in children [3]. While first-line and second-line antibiotic treatment recommendations have been developed to treat children with AOM [3], the infection can persist in some cases, termed AOM treatment failure.

AOM treatment failure can be defined as: (i) the persistence of clinical and otoscopic signs of AOM, despite one or several courses of antibiotic therapy; (ii) the recurrence of AOM in a short period of time after the antibiotic course is finished; (iii) intracranial/intratemporal complications during AOM; or (iv) continued tympanic membrane inflammation or acute symptoms, despite antibiotic therapy. The main causes of AOM treatment failure in children are Eustachian tube dysfunction, immaturity of the immune system in childhood, inappropriate administration of an antibiotic by parents, anatomy, previous or concurrent viral infection and antibiotic-resistant bacteria, among others [4]. Unfortunately, the prevalence of antibiotic-resistant bacteria typically causative of AOM is increasing and has become a major concern for effective treatment.

The major bacterial pathogens responsible for AOM are *Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis* (for recent reviews see [5,6]). Due to the excessive and improper use of antibiotics to treat infections, there has been a global increase in the prevalence of antibiotic-resistant bacterial strains, the severity of which can vary based on geographical location [7]. In Poland, data from the National Referral Center of Microbial Resistance (Krajowy Ośrodek Referencyjny ds. Lekowrażliwości Drobnoustrojów) have shown increased resistance of the major causative bacterial agent of AOM (*S. pneumoniae*) to penicillin

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and other antibiotics [8–12], with the first multidrug-resistant clones of *S. pneumoniae* described in 1978 [13]. However, whether the bacterial strains found in children with AOM treatment failure are resistant to multiple types of antibiotics remains unclear.

In this prospective study, we aimed to identify the most commonly isolated bacterial strain in children with AOM in Poland. We performed a bacteriological analysis of the fluid isolated from the middle ear in children with AOM whose antibiotic treatment was unsuccessful. We also determined the antibiotic resistance profile of the most frequently isolated strain. Together, the results of our study will help us to understand the current failure of empirical antibiotic therapy in the treatment of AOM.

2. Materials and methods

2.1. Patients

In this prospective study, we examined 157 children aged from 6 months to 7 years (96 boys and 61 girls, with a mean age of 3 years and 3 months). All children were admitted to the Department of Otolaryngology, Children's Hospital in Warsaw, Poland between 2010 and 2013 because of the failure of oral antibiotic therapy for treatment of AOM.

AOM antibiotic treatment failure was defined as: (i) the persistence of clinical and otoscopic signs of AOM (i.e. fever, severe earache, bulging tympanic membrane) within 2–10 days of an initial diagnosis and after the prescription of initial or delayed antibiotic therapy and (ii) the recurrence of AOM after 6–30 days of finishing the antibiotic course. First-line and second-line antibiotic treatment was administered for a period of 2–10 days and based on recommendations described by Rosenfeld [3] as follows: amoxicillin (80–90 mg/kg/day), amoxicillin-clavulanate (90 mg/kg/day of amoxicillin with 6.4 mg/kg/day of clavulanate), cefuroxime (30 mg/kg/day) and clindamycin (30–40 mg/kg/day). Children were referred for myringotomy due to: (i) increased clinical symptoms (fever, severe earache), and the observed presence of a bulging tympanic membrane with pus in the tympanic cavity upon otoscopic and microscopic examination; and (ii) AOM antibiotic treatment failure.

Children with spontaneous perforation and a previously inserted ventilatory tube were excluded from this study. All children included in this study were immunized with the *Haemophilus influenzae* type B (Hib) vaccine. None of the children were immunized with the 7-valent pneumococcal conjugate vaccine (PCV-7) (Prevenar, Wyeth Pharmaceuticals), which was introduced for pediatric use in 2000 (for a review of the global data on this vaccine see [14]). All children attended a day care center or nursery school

2.2. Middle ear fluid (MEF) sample collection and analysis

Myringotomy was performed in all children, and the MEF was collected for bacteriological examination. Briefly, the external ear canal was rinsed for 1 min with 70% ethanol. Then, under otomicroscopic visual control, a sterile suction trap was used to aspirate MEF samples by myringotomy through the intact tympanic membranes. All MEF samples were transferred to the Department of Laboratory Diagnostics Children's Hospital in Warsaw, Poland within 2–6 h for plating at room temperature.

Pathogenic bacteria in the MEF were identified using standard methods (www.eucast.org). Briefly, MEF was plated onto the following: Columbia agar with 5% sheep blood; Brain Heart Infusion (BHI) agar (for cultivation of *H. influenza*); MacConkey agar (for cultivation of Gram-negative bacilli); and Chapman agar (for cultivation of *S. aureus*). The Columbia agar, MacConkey agar and Chapman agar were incubated at 36–37 °C for 24 h. The BHI agar was incubated at 36–37 °C with 5% CO_2 for 24 h. After the incubation period, bacterial growth was determined using diagnostic discs and biochemical methods.

Antibiotic sensitivity of *S. pneumoniae* was determined using standard disk and strip tests for oxacyline, erythromycin, clindamycin and sulfamethoxazole-trimetopim. In the case of oxycyline antibiotic-resistant strains, the sensitivity to cefotaxime was also evaluated. Definitions of antimicrobial susceptibility were based on laboratory standards. Susceptibility of isolated *S. pneumoniae* was determined by a disk diffusion technique containing different concentration gradients for the following antibiotics: penicillin, ampicillin, cefotaxime and ceftriaxone. Non-susceptibility to three or more antibiotic classes was considered a multidrug-resistant strain. *S. pneumoniae* isolates were classified as penicillin-susceptible (S, MIC $\leq$ 0.06 μg/ml), penicillin intermediate-resistant (I, MIC $>$ 0.06–2.0 μg/ml) and penicillin-resistant (R, MIC $>$ 2.0 μg/ml).

2.3. Ethical approval

The parents of the study subjects were informed of the method and purpose of the study and their consent was obtained. The study was approved by the Bioethics Committee in Warsaw, Poland.

3. Results

3.1. Common bacterial strains found in the MEF of children with AOM treatment failure

Positive bacterial cultures were obtained in 104 children (66.2%) with AOM, while no bacterial etiological agents were identified in the remaining 33.8% of AOM children. A total of 131 different bacterial strains were isolated as two different bacteria were isolated in 27 children with AOM. The most frequently isolated pathogens were: *S. pneumoniae* (39.69%), *H. influenzae* (16.03%), *S. aureus* (16.03%), *S. haemolyticus* (6.9%) *and S. pyogenes* (5.34%); other bacteria were identified in 16.01% of all cases (Table 1).

3.2. Antibiotic resistance of the most commonly isolated bacterial strain

Because *S. pneumoniae* was isolated most frequently (52/131, 39.69% of all strains), we focused on analyzing the antibiotic

Table 1
Bacteriological flora isolated from the middle ear fluid of children with treatment failure for acute otitis media.

Bacteria	Number of isolated bacteria [%]
Streptococcus pneumoniae	52 [39.69%]
Staphylococcus aureus	21 [16.03%]
Haemophilus influenzae	21 [16.03%]
Staphylococcus haemolyticus	9 [6.9%]
Streptococcus alpha-hemolytic	7 [5.34%]
Streptococcus pyogenes	7 [5.34%]
Streptococcus beta-hemolytic	3 [2.29%]
Escherichia coli ESBL (-)	3 [2.29%]
Enterobacter cloacae	2 [1.52%]
Klebsiella pneumoniae ESBL (-)	2 [1.52%]
Proteus mirabilis	1 [0.76%]
Acinetobacter	1 [0.76%]
Streptococcus mitis	1 [0.76%]
Pseudomonas aeruginosa	1 [0.76%]
Total strains	131 [100%]