Increased recovery of *Moraxella catarrhalis* and *Haemophilus influenzae* in association with group A β -haemolytic streptococci in healthy children and those with pharyngo-tonsillitis

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The inflamed tonsils harbour numerous types of bacteria, alone or in combination with group A β -haemolytic streptococci (GABHS). The cohabitation of the tonsils by GABHS and certain other bacterial species may contribute to the inflammatory process and the failure of penicillin therapy. This study evaluated the recovery of *Moraxella catarrhalis*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* in association with GABHS in healthy children and those with acute pharyngo-tonsillitis (APT). Pharyngo-tonsillar cultures were obtained from 548 children with APT and 866 healthy children. GABHS was recovered from 112 (20.4%) children with APT. Of the 114 *H. influenzae* isolates, 32 were recovered in association with GABHS (29% of all patients who had GABHS) and 82 were isolated without GABHS (19%) ($P=0.0267$). Of the 69 *M. catarrhalis* isolates, 25 were recovered in association with GABHS (22% of all patients who had GABHS) and 44 were isolated without GABHS (10%) ($P=0.0012$). In contrast, there was no association between the isolation of GABHS and the recovery of *Staph. aureus* or *Strep. pneumoniae*. GABHS was recovered from 104 (12%) healthy children. Of the 69 *M. catarrhalis* isolates, 24 were recovered in association with GABHS (23% of all patients who had GABHS) and 80 were isolated without GABHS (10%) ($P=0.006$). There was no association between the isolation of GABHS and the recovery of *H. influenzae*, *Staph. aureus* or *Strep. pneumoniae*. This study demonstrates an association between the recovery of GABHS and *H. influenzae* and *M. catarrhalis* from pharyngo-tonsillar cultures of patients with APT and *M. catarrhalis* from pharyngo-tonsillar cultures of healthy children.

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INTRODUCTION

Acute pharyngo-tonsillitis (APT) is a common infection, especially in children and young adults. The diagnosis of bacterial APT generally requires the consideration of group A β -haemolytic streptococci (GABHS) infection. However, numerous other bacteria (including *Moraxella catarrhalis* and *Haemophilus influenzae*) can be recovered from these tonsils alone or in combination with GABHS.

GABHS is a common colonizer of the pharynx of children. These individuals are sometimes labelled as 'carriers' of GABHS. Even though the carriage of GABHS is generally not associated with any adverse effects, the recovery of GABHS from a child with symptoms of APT may confirm the clinical diagnosis. Furthermore, individuals colonized with GABHS can serve as a source of spread of this organism to their contacts. In addition to GABHS, numerous other aerobic

and anaerobic bacteria, alone or in combination with GABHS, can be recovered from the pharynx of children (Brook, 2005).

Several studies suggest that the colonization of the tonsils by GABHS and certain other aerobic and anaerobic bacterial species may contribute to the inflammatory process and the failure of penicillin therapy (Brook, 2005). A mutual symbiotic enhancement of growth of GABHS in the presence of other aerobic and anaerobic bacteria has been demonstrated in an animal model (Brook & Gillmore, 1996). Such a synergistic relationship may also exist between these organisms and GABHS in patients with tonsillitis. An example of a potential synergistic relationship is the ability of *M. catarrhalis* to increase the adherence of GABHS to human epithelial cells through species-specific co-aggregation (Lafontaine *et al.*, 2004).

Several of the organisms that are isolated from the tonsils can produce the enzyme β -lactamase. These include *M. catarrhalis*, *H. influenzae* and *Staphylococcus aureus*. This

Abbreviations: APT, acute pharyngo-tonsillitis; BLPB, β -lactamase-producing bacteria; GABHS, group A β -haemolytic streptococci.

enzyme can inactivate penicillin and contribute to the failure of this agent to eradicate GABHS from infected tonsils (Brook, 1984).

This study investigated whether the recovery of *M. catarrhalis*, *H. influenzae*, *Staph. aureus* and *Streptococcus pneumoniae* is associated with GABHS in healthy children and those with APT.

METHODS

Patients. The population studied was middle class, residing in suburban locations in the vicinity of Washington, DC. Patients seen consecutively for APT and those seen consecutively for their annual physical examination were included in the study. APT was defined as acute onset of sore throat plus at least one of the following: anterior cervical adenitis, temperature >38.3 °C (101 °F), pharyngeal or tonsillar exudates, or pharyngeal injection. The study was granted an Institutional Review Board approval.

A total of 548 children with APT were evaluated. The median age of the children was 7 years and 4 months (range, 2.5–14 years). Past medical history, including antimicrobial therapy and day-care attendance, was obtained by using a questionnaire and reviewing medical records. Those who received antimicrobial therapy or who had suffered from GABHS APT in the previous 3 months were excluded.

A total of 866 healthy children were evaluated as control subjects. The median age of the children was 6 years and 10 months (range, 2.5–14 years). Past medical history, including antimicrobial therapy and day-care attendance, was obtained by using a questionnaire and reviewing medical records. Excluded were those who had received antimicrobial therapy or suffered from GABHS APT in the previous 3 months.

Bacteriological methods. Pharyngo-tonsillar cultures were obtained from all children with a sterile cotton swab. The swab was placed in a transport system for aerobic bacteria (Culturette, Marion Scientific) and inoculated within 24 h of collection. Sheep blood (5%), chocolate and MacConkey agar plates were inoculated for the isolation of aerobic organisms. Plates were incubated at 37 °C aerobically (MacConkey) and in 5% carbon dioxide (blood and chocolate), and examined at 24 and 48 h. The organisms were identified by conventional methods (Murray *et al.*, 1995). GABHS were identified by determining bacitracin sensitivity and by serologic grouping by Phadebact coagglutination (Pharmacia Diagnostics).

β -Lactamase activity was determined by chromogenic cephalosporin analogue 87/312 methodology (O’Callaghan *et al.*, 1972). Statistical analysis was done using Fisher’s exact test. *P* values were two-sided. Comparisons were made using StatXact software.

RESULTS

Children with APT

GABHS was recovered from 112 (20.4 %) children. GABHS was isolated in the absence of *M. catarrhalis*, *H. influenzae*, *Staph. aureus* and *Strep. pneumoniae* in 34 instances, in association with one of the other isolates in 64 cases, with two others in ten, and with three others in four children.

Thirty-two of 114 *H. influenzae* isolates were recovered in association with GABHS (29 % of all patients who had GABHS) and 82 were isolated without GABHS (19 % of all patients who had no GABHS) (*P*=0.0267). Twenty-five of the 69 *M. catarrhalis* isolates were recovered in association with GABHS (22 % of all patients who had GABHS) and 44 were isolated without GABHS (10 % of all patients who had no GABHS) (*P*=0.0012) (Table 1). In contrast there was no association between the isolation of GABHS and the recovery of *Staph. aureus* or *Strep. pneumoniae*.

One hundred and ninety-five β -lactamase-producing bacteria (BLPB) were recovered from 162 (30 %) patients. These included all isolates of *M. catarrhalis* and *Staph. aureus*, and 40 (35 %) of *H. influenzae*.

Healthy children

GABHS was recovered from 104 (12 %) of the 866 children. GABHS was isolated without any of the other four organisms in 32 instances, in association with one of the other isolates in 62 cases, with two others in seven, and with three others in four children.

Of the 69 *M. catarrhalis* isolates, 24 were recovered in association with GABHS (23 % of all patients who had GABHS) and 80 were isolated without GABHS (10 % of all patients who had no GABHS) (*P*=0.006) (Table 2). In

Table 1. Number of isolates recovered with and without GABHS in 548 children with pharyngo-tonsillitis

The percentage of patients in each group is shown in parentheses.

Co-isolates	Recovered in patients with GABHS (n=112)	Recovered in patients without GABHS (n=436)	Total (n=548)
<i>H. influenzae</i> non-type b	32 (29%)*	82 (19%)	114
<i>M. catarrhalis</i>	25 (22%) [†]	44 (10%)	69
<i>Staph. aureus</i>	19 (17%)	67 (15%)	86
<i>Strep. pneumoniae</i>	20 (18%)	68 (16%)	88
Total	96	261	357

**P*=0.0267.

[†]*P*=0.0012.

Table 2. Number of isolates recovered with and without GABHS in 866 healthy children

The percentage of patients in each group is shown in parentheses.

Co-isolates	Recovered in patients with GABHS (n = 104)	Recovered in patients without GABHS (n = 762)	Total (n = 866)
<i>H. influenzae</i> non-type b	23 (22 %)	126 (17 %)	149
<i>M. catarrhalis</i>	24 (23 %)*	80 (10 %)	104
<i>Staph. aureus</i>	11 (11 %)	104 (14 %)	115
<i>Strep. pneumoniae</i>	10 (10 %)	73 (10 %)	83
Total	68	383	451

* $P = 0.006$

contrast there was no association between the isolation of GABHS and the recovery of *H. influenzae*, *Staph. aureus* or *Strep. pneumoniae*.

Two hundred and seventy-one BLPB were recovered from 221 (26 %) patients. These included all isolates of *M. catarrhalis* and *Staph. aureus*, and 52 (35 %) of *H. influenzae*.

DISCUSSION

This study demonstrates an association between the recovery of GABHS and *H. influenzae* and *M. catarrhalis* from pharyngo-tonsillar cultures of children with APT, and *M. catarrhalis* from pharyngo-tonsillar cultures of healthy children. *H. influenzae* and *M. catarrhalis* have been recovered in 10–20 % of acutely (Brook, 1985), recurrently (Stjernquist-Desatnik *et al.*, 1990) and chronically (Brook *et al.*, 1995) inflamed tonsils. However, this study is the first to associate their isolation in conjunction with the recovery of GABHS.

The increased isolation of *H. influenzae* (in APT only) and *M. catarrhalis* in association with GABHS may be due to a synergistic relationship between these organisms (Brook & Gillmore, 1996; Lafontaine *et al.*, 2004). The ability of *H. influenzae* and *M. catarrhalis* to produce the enzyme β -lactamase may also make them, as well as GABHS, more resistant to eradication by penicillin and contribute to failure of penicillin therapy. This may be due to the ability of β -lactamase to inactivate the penicillin in the tonsillar tissues (Brook, 1984).

We have previously demonstrated an association between the recovery of these, as well as other BLPB, and an increased failure of penicillin to eradicate GABHS (Brook, 1985). We evaluated 98 children who had acute tonsillitis due to GABHS and were treated for 10 days with orally administered penicillin. On the basis of bacteriologic results, 62 patients were considered 'cured' (group A) and 36 'failed' (group B) following therapy. Before therapy, 18 isolates of BLPB were detected in 16 (26 %) children in group A; after therapy 30 BLPB were detected in 19 (30 %) children. In contrast, before therapy, 40 BLPB were recovered from 25

(69 %) children in group B; this number increased to 62 BLPB in 31 (86 %) of those children.

An indirect support for the potential synergistic relationship between GABHS and *H. influenzae* and *M. catarrhalis* is the better clinical efficacy, as compared to penicillin, of second-generation extended-spectrum, and third-generation cephalosporins (Casey & Pichichero, 2004), as well as the combination of amoxycillin and clavulanate (Brook, 1989; Kaplan & Johnson, 1988), in eradicating GABHS pharyngo-tonsillitis. The superior efficacy of these agents compared to penicillin may be due to their activity against GABHS, as well as β -lactamase-producing *H. influenzae* and *M. catarrhalis*.

Further studies are warranted to evaluate the nature of the interactions between GABHS and *H. influenzae* and *M. catarrhalis*, and whether antimicrobials active against these organisms are more effective in eradication of GABHS infection.

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