Recovery of interfering and β -lactamase-producing bacteria from group A β -haemolytic streptococci carriers and non-carriers

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The purpose of this study was to compare the frequency of recovery of aerobic and anaerobic organisms with interfering capability against group A β -haemolytic streptococci (GABHS) and β -lactamase-producing bacteria (BLPB) from the tonsils of GABHS carriers and non-carriers. The presence of aerobic and anaerobic bacteria capable of such interference *in vitro* was evaluated in cultures obtained from the tonsils of 20 healthy children who were non-GABHS carriers and 20 who were GABHS carriers, and also from 20 children who were asymptomatic after completing a course of penicillin for acute GABHS pharyngo-tonsillitis (PT) and were non-GABHS carriers and 20 who were GABHS carriers. In healthy children, 32 interfering isolates were recovered from 16 non-GABHS carriers (1.6 per child) and 13 were isolated from 7 GABHS carriers (0.65 per child) ($P < 0.001$). In children who had suffered acute GABHS PT, 26 interfering organisms were recovered from 15 non-GABHS carriers (1.3 per child) and 8 were isolated from 5 GABHS carriers (0.4 per child) ($P < 0.005$). In healthy children, 13 BLPB were recovered from 5 non-GABHS carriers and 13 were isolated from 6 GABHS carriers. In children who had suffered acute GABHS PT, 14 BLPB were recovered from 5 (25 %) non-GABHS carriers and 32 were isolated from 17 (85 %) GABHS carriers ($P < 0.05$). It was demonstrated in this study that there was a higher rate of recovery of aerobic and anaerobic organisms capable of interfering with GABHS in non-GABHS carriers than in GABHS carriers. This was observed in all GABHS non-carriers and included healthy children as well as those recently treated for symptomatic GABHS PT with penicillin that failed to eradicate GABHS. A higher rate of recovery of BLPB was observed only in GABHS carriers who were treated with penicillin for GABHS PT.

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INTRODUCTION

Group A β -haemolytic streptococci (GABHS) (*Streptococcus pyogenes*) are the most common cause of bacterial pharyngo-tonsillitis (PT) (Bisno, 1991). They are also the main cause of acquired heart disease among children throughout the world, and increasingly they are a major cause of deaths attributable to bacterial sepsis in all age groups (O'Brien *et al.*, 2002; Cockerill *et al.*, 1997).

Children are the major reservoir of GABHS and are the target population for PT, as well as its suppurative and non-suppurative complications (O'Brien *et al.*, 2002). Carriers of GABHS harbour the organism in their nose or throat but display no symptoms of acute infection. They represent the pool from which patients with severe invasive disease may acquire their infections (Cockerill *et al.*, 1997). The reasons that lead to the GABHS carrier state are not yet established.

Penicillin therapy is only 30 % effective in eradicating the carrier state (Tanz *et al.*, 1985), as compared to over 90 % for clindamycin therapy (Tanz *et al.*, 1991). The presence of β -lactamase-producing bacteria (BLPB) in the tonsils, which protect GABHS from penicillin by inactivating the antibiotic, was offered as one of the explanation for penicillin failure in eradication of an acute GABHS tonsillitis (Brook, 1984). The presence of BLPB in the tonsillar flora of GABHS carriers was not previously studied.

An inverse relationship has been found between the presence of aerobic and anaerobic organisms with interfering capability against GABHS and the isolation of this organism from the tonsils (Brook & Gober, 1999). These bacteria have been shown *in vitro* to compete and thus interfere with GABHS growth (Crowe *et al.*, 1973). The prevalence of these interfering organisms has never been evaluated in GABHS carriers.

The purpose of the present study was to compare the frequency of recovery of aerobic and anaerobic organisms with interfering capability against GABHS as well as BLPB

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

from the tonsils of GABHS carriers and non-carriers. The carriers of GABHS included healthy children that were seen for their annual physical examination and those recently treated for symptomatic GABHS PT with penicillin that failed to eradicate the GABHS.

METHODS

Consecutively examined children seen in the outpatient clinic were included in the study. Only one child per household was included. A child was regarded as a carrier of GABHS when they were clinically asymptomatic and 10 colonies or more per blood agar plate of these organisms were isolated from a culture of their tonsils. Two groups of children were included in the study: group 1 consisted of healthy children seen for their annual physical examination. Included were the first 20 children who were non-GABHS carriers and the first 20 who were GABHS carriers. Group 2 included clinically asymptomatic children who initially presented with PT and positive cultures for GABHS, completed a 10 day course of oral penicillin-V, and were seen at a follow-up visit 14–21 days after initiation of antimicrobial therapy. Included were the first 20 children who were non-GABHS carriers and the first 20 who continued to harbour GABHS. A total of 80 children (43 boys) were included, with a mean age of 7·8 years (range 4·5–15 years). Age and sex distribution was similar in all groups. Excluded were those who received antimicrobial therapy in the previous 2 months, had any acute or chronic medical or dental problem, or suffered from GABHS PT in the previous 6 months. The protocol was approved by the Institutional Review Board, Georgetown University School of Medicine.

Both tonsillar surfaces were swabbed with two sterile cotton swabs. Aerobic cultures were obtained by placing a swab in modified Stuart's bacterial transport system (Baltimore Biological Laboratories). Cultures for anaerobes were obtained by introducing a swab into anaerobic transport medium (Port-A-Cul; Becton Dickinson Labware). The specimens were inoculated within 2 h of collection.

Sheep's blood (5%), chocolate and MacConkey agar plates were inoculated for the isolation of aerobic organisms. The culture plates were incubated aerobically at 37 °C (MacConkey agar) and under 5% carbon dioxide (blood and chocolate agars), and they were examined at 24 and 48 h. For the recovery of anaerobic bacteria, the specimens were inoculated onto pre-reduced vitamin K₁-enriched *Brucella* blood agar, blood agar that contained kanamycin and vancomycin, and an aerobic

blood plate that contained phenylethyl alcohol and enriched thioglycolate broth. These media were immediately incubated in GasPak jars (Baltimore Biological Laboratories) at 37 °C, and examined after 48 and 96 h of incubation at 37 °C. All types of colonies on each plate were isolated. Aerobic and anaerobic bacteria were identified by published methods (Summanen *et al.*, 1995; Murray *et al.*, 2003). β -Lactamase activity was determined for five colonies of each of the anaerobic and aerobic isolates by using a cefinaz disk (Baltimore Biological Laboratories).

Testing for interference. The inhibitory activity of five separate colonies from all aerobic and anaerobic isolates was evaluated by individually testing against one strain of a recent clinical isolate of *S. pyogenes*, using a Steer's steel pin replicator as described by Grahn *et al.* (1983). In brief, minidrops of exponential-phase broth cultures of the isolates were transferred with the pin replicator to vitamin K₁-enriched *Brucella* blood agar plates and allowed to dry for 15 min at room temperature. A sample of an exponential-phase broth culture of the *S. pyogenes* strain was applied adjacent to each of the isolated strains, and the plates were incubated in 5% carbon dioxide or anaerobically at 37 °C for 48 h. Bacterial interference was defined as any reproducible inhibition of growth. Degrees of inhibition varied from complete absence of growth to a narrow zone of poor growth along the proximal area of the colony. Statistical significance was calculated by Fishers exact test (two-sided) unadjusted.

RESULTS

Interfering Organisms

Children seen for annual examination (group 1). Thirty-two interfering isolates (including α -streptococci, non-haemolytic streptococci, *Prevotella* spp. and *Peptostreptococcus* spp.) were recovered from sixteen (80%) non-GABHS carriers (1·6 per child) and thirteen were isolated from seven (35%) GABHS carriers (0·65 per child) ($P < 0\cdot001$, when comparing the proportion of children with interfering organisms) (Table 1).

Children seen after having treatment with penicillin for acute GABHS tonsillitis (group 2). Twenty-six interfering isolates (including bacteria as described for group 1) were recovered from fifteen (75%) non-GABHS carriers (1·3 per child) and eight were isolated from five

Table 1. Number of isolates (percentage of children) with interfering capabilities against GABHS recovered from the surface of tonsils of non-GABHS carriers and GABHS carriers

Organism	Group 1: healthy children		Group 2: children after having treatment with penicillin for acute GABHS PT	
	Non-GABHS carriers (n=20)	GABHS carriers (n=20)	Non-GABHS carriers (n=20)	GABHS carriers (n=20)
α -Haemolytic streptococci	9 (45)	2 (10)	7 (35)	1 (5)
Non-haemolytic streptococci	5 (25)	3 (15)	4 (20)	2 (10)
<i>Prevotella</i> spp.	10 (50)	4 (20)	8 (40)	3 (15)
<i>Peptostreptococcus</i> spp.	8 (40)	4 (20)	7 (35)	2 (10)
Total	32	13*	26	8†

* $P < 0\cdot001$.

† $P < 0\cdot005$.

(25 %) GABHS carriers (0.4 per child) ($P < 0.005$, when comparing the proportion of children with interfering organisms) (Table 1).

BLPB

Children seen for annual examination (group 1). Thirteen BLPB (including *Staphylococcus aureus*, *Moraxella catarrhalis*, *Haemophilus influenzae* non-type b, *Haemophilus parainfluenzae*, *Prevotella* spp., *Porphyromonas* spp. and *Fusobacterium* spp.) were recovered from five (25 %) non-GABHS carriers (0.65 per child) and thirteen were isolated from six (30 %) GABHS carriers (0.65 per child) (Table 2).

Children seen after having treatment with penicillin for acute GABHS tonsillitis (group 2). Fourteen BLPB (including bacteria as described for group 1) were recovered from five (25 %) non-GABHS carriers (0.7 per child) and thirty-two were isolated from seventeen (85 %) GABHS carriers (1.6 per child) ($P < 0.05$, when comparing the proportion of children with BLPB) (Table 2).

DISCUSSION

We observed a higher rate of recovery of aerobic and anaerobic organisms capable of interfering with the *in vitro* growth of GABHS in non-GABHS carriers than GABHS carriers. This was observed in all GABHS non-carriers and included healthy ones that were seen for their annual physical examination, as well as those recently treated for symptomatic GABHS PT with penicillin that failed to eradicate the GABHS. In contrast, we found a higher rate of recovery of BLPB only in GABHS carriers who were treated with penicillin for an acute GABHS PT.

These findings shed light on the unique bacterial flora of non-GABHS carriers and GABHS carriers, those who had

no recent PT (group 1) and those who did suffer from GABHS PT and were recently treated with penicillin (group 2).

The association between the presence of high number of BLPB and colonization of the tonsils by GABHS in patients who were recently treated with penicillin, is explained by the protection provided to GABHS from the antibiotic (Brook, 1984). Recently treated patients who were not carriers were less likely to harbour BLPB. These findings are in concert with the clear association that has been established in the therapy of GABHS PT between the failure of patients to respond to penicillin, and the pre-existence of BLPB (Brook, 1984). Over 3/4 of tonsils removed due to recurrent tonsillitis, harbour BLPB and free β -lactamase was detected in the core of the majority of those tonsils.

There is an intricate balance in the oropharynx between potential pathogens, including GABHS and interfering organisms. The relative paucity of interfering organisms in GABHS carriers occurred in healthy children as well as children recently treated for PT. The protective ability of interfering organisms may therefore play a role in the prevention of the carrier state. Prevention of upper respiratory bacterial infections is partially achieved because of the ability of members of the normal oropharyngeal flora to deter colonization and subsequent infection by those pathogens. The oropharynx flora of individuals who are not GABHS tonsillitis prone contains more organisms that are capable of interfering with the *in vitro* growth of potential pathogens than those who are GABHS tonsillitis prone (Brook & Gober, 1995, 1999). Only about 30 % of patients who suffer from recurrent GABHS tonsillitis are colonized with organisms that are capable of interfering with GABHS (Brook & Gober, 1995, 1999). On the other hand, 85 % of individuals who stay GABHS PT free are colonized by those protective organisms. This phenomena of protection is called bacterial interference (Brook, 2005; Grahm *et al.*,

Table 2. Number of BLPB recovered from the surface of tonsils of non-GABHS carriers and GABHS carriers

Entries are expressed as the number of isolates of the species (number of those that produced β -lactamase).

Organism	Group 1: healthy children		Group 2: children after having treatment with penicillin for acute GABHS PT	
	Non-GABHS carriers (n = 20)	GABHS carriers (n = 20)	Non-GABHS carriers (n = 20)	GABHS carriers (n = 20)
<i>Staphylococcus aureus</i>	1 (1)	3 (3)	3 (3)	7 (7)
<i>Moraxella catarrhalis</i>	2 (2)	5 (5)	3 (3)	9 (9)
<i>Haemophilus influenzae</i> non-type b	2 (1)	2 (1)	5 (1)	7 (3)
<i>Haemophilus parainfluenzae</i>	2 (1)	2 (0)	1 (0)	2 (1)
<i>Fusobacterium</i> spp.	7 (2)	7 (1)	6 (3)	8 (3)
<i>Prevotella</i> spp.	20 (4)	20 (2)	20 (4)	20 (7)
<i>Porphyromonas</i> spp.	7 (2)	7 (1)	5 (0)	6 (2)
Total	41 (13)	46 (13)	43 (14)	59 (32)*

* $P < 0.05$.

1983). The present study suggests that the *in vitro* interference phenomena also may have *in vivo* correlates in children who are GABHS carriers.

The normal flora interferes with colonization and subsequent infection by pathogens through various mechanisms. These include competition on nutritional substances, and the production of antibiotic-like substances that kill other bacteria called bacteriocins (Brook, 2005; Grahn *et al.*, 1983; Walls *et al.*, 2003). The major protective organisms are α - and γ -haemolytic streptococci, *Peptostreptococcus* spp. and *Prevotella* spp. These organisms play a homeostatic role by colonizing the tonsils in sufficient numbers that they prevent colonization and infection by GABHS.

Therapeutic colonization of the nasopharynx with interfering bacteria was studied by Roos *et al.* (1996) who colonized 112 children with repeated tonsillitis with either α -haemolytic streptococci or placebo. Recurrences occurred in 1 of 51 children (2%) in the α -haemolytic streptococci group and 14 of 61 children (23%) in the placebo group.

Antibiotics that possess broad activity against interfering organisms may reduce their numbers in the oral flora (Brook & Gober, 1995; Brook, 2001). Pichichero *et al.* (1999) found that the GABHS carrier rate is higher following treatment of acute GABHS PT with penicillin (11.3%), than with an oral cephalosporin (4.3%) ($P < 0.001$). The cephalosporin group of antibiotics have been successful in eradicating GABHS better, and in some instances much faster, than penicillin (Casey & Pichichero, 2004). Over 50 studies show that all classes and types of cephalosporins have a much higher success rate in eradicating GABHS than penicillin (Casey & Pichichero, 2004). The explanation for the ability of cephalosporins to perform so well is that in addition to their efficacy against GABHS they are less effective against the interfering organisms (Brook & Foote, 2005). They are less active than penicillins against anaerobic bacteria (Finegold & Sutter, 1976) and α -haemolytic streptococci (Brook & Foote, 2005).

Our data suggest that bacterial interference and β -lactamase production may play a role in the presence of the GABHS carrier state. Further studies are warranted to evaluate how modulation of the normal flora can prevent the GABHS carrier state.

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