Evaluation of potential factors contributing to microbiological treatment failure in *Streptococcus pyogenes* pharyngitis

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BACKGROUND: A cohort study of children with pharyngitis aged two to 16 years was conducted to assess the role of microbial and host factors in group A beta-hemolytic streptococcus (GABHS) microbiological treatment failure.
METHODS: GABHS-infected children had pharyngeal swabs repeated two to five days after completing a 10-day course of penicillin V. M and T typing, and pulsed field gel electrophoresis were performed on the isolates, and the isolates were evaluated for tolerance. Patient characteristics and clinical features were noted and nasopharyngeal swabs for respiratory viruses were taken at enrolment.
RESULTS AND CONCLUSIONS: Of 286 patients enrolled, 248 (87%) could be evaluated. GABHS was cultured from 104 patients (41.9%), of whom 33 (33.7%) had microbiological treatment failures on follow-up. Although there was a trend toward failure for younger children (mean 6.5±2.4 years versus 7.3±2.4 years, P=0.07) and M type 12 (24% versus 10%, P=0.08), no factors were associated with treatment failure.

Key Words: *Pharyngitis;* Streptococcus pyogenes*; Respiratory viruses; Treatment failure*

Évaluation des facteurs potentiels contribuant à l'échec microbiologique dans la pharyngite à *Streptococcus pyogenes*

HISTORIQUE : Étude de cohorte auprès d'enfants souffrant de pharyngite, âgés de 2 à 16 ans, effectuée dans le but d'évaluer le rôle des antibiotiques et des facteurs liés à l'hôte dans l'échec du traitement antibiotique contre le streptocoque bêta-hémolytique du groupe A (GABHS).
MÉTHODES : Des enfants infectés au GABHS ont subi des cultures de gorge à répétition, deux à cinq jours après une antibiothérapie de dix jours avec pénicilline V. Le typage M et T et l'électrophorèse sur champ pulsé ont été appliqués aux isolats et ces derniers ont été évalués sur le plan de la tolérance. Les caractéristiques des patients et les caractéristiques cliniques ont été notées et des échantillons naso-pharyngés ont été prélevés pour frottis afin de déceler la présence de virus respiratoires au moment de l'admission à l'étude.
RÉSULTATS ET CONCLUSION : Parmi les 286 patients inscrits, 248 (87 %) ont pu être évalués. Le GABHS a été mis en culture chez 104 patients (41,9 %), dont 33 (33,7 %) ont présenté des échecs thérapeutiques au moment du suivi. Malgré une tendance à l'échec thérapeutique chez les enfants plus jeunes (moyenne 6,5 ± 2,4 ans vs 6,3 ± 2,4 ans; $p = 0,07$) et un typage M 12 (24 % vs 10 %; $p = 0,08$), aucun facteur n'a été associé à l'échec thérapeutique.

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Pharyngeal group A beta-hemolytic streptococcus (GABHS) microbiological treatment failure has been reported in 2.5% to 37% of children with GABHS pharyngitis (1,2), although most studies find treatment failure in the range of 10% to 15% (3). Treatment failure is defined as the detection of GABHS of the same serotype, with or without symptoms of pharyngitis, after recent completion of appropriate antibiotic therapy. Follow-up cultures are usually performed five to 10 days after completing antibiotics to confirm eradication (1,4,5). Symptomatic treatment failure in GABHS pharyngitis has a greater risk of acute rheumatic fever and other complications than asymptomatic treatment failure (6,7). Recent concerns regarding outbreaks of rheumatic fever (8) and occasional severe suppurative complications (9) have led some authorities to suggest attempted eradication in some patients, particularly when associated with continued symptoms (10).

A variety of mechanisms have been proposed for treatment failures, including: reinfection through various means, including fomites (11); lack of compliance (12); streptococcal tolerance to penicillin (13); early initiation of antibiotics resulting in inadequate immune response (4,14); lack of protective microflora or its inadvertent eradication (15,16); and copathogenicity of beta-lactamase-producing flora (BLPF), such as anaerobes (1,2), with their resulting implications for the choice of antibiotic treatment (3).

Other factors may also contribute to treatment failure. These include host and treatment factors, such as demographic features, and clinical signs and symptoms at presentation. Organism characteristics, such as microbial load and serotype, may be important. Serotype has been used to distinguish treatment failure from new infection but has not been evaluated as an independent risk factor. Pulsed field gel electrophoresis (PFGE) has not been employed in past treatment failure studies to determine the relatedness of initial and follow-up strains but has been shown to be useful in distinguishing new infections from relapses involving the same organism (17). Also, although the influence of coexistent bacterial flora has been considered, there are other pharyngeal organisms to examine.

A hypothesis that has not been considered is a possible association between respiratory viruses and GABHS treatment failure. There is a temporal similarity in the occurrence of the two infections: streptococcal pharyngitis is most common during fall and winter months in the northern latitudes (18), and respiratory viral infections have a similar season. Coisolation of GABHS with a virus has been reported to occur in 8% of paediatric pharyngitis cases, although it is unclear whether this actually represents infection (19). Viral infections have direct effects on respiratory epithelial cells, including ciliary impairment (20) and increased bacterial adherence (21). Viruses may also alter the host immune response (22,23). Both the impact on respiratory epithelial cells and the alteration of host immune response may not only increase the risk of infection, but may also impede bacterial clearance despite therapy. Respiratory virus isolation, coexisting with bacterial acute otitis media (AOM), has been associated with both persistent symptoms of AOM (24) and persistent isolation of bacteria in the middle ear fluid after institution of antibiotic therapy (25-27). Given the parallels of AOM and GABHS pharyngitis in seasonality, in apparent relationship to respiratory viruses and in anatomic proximity, a similar relationship between viral coisolation and failure to eradicate GABHS may also be found in GABHS pharyngitis.

The objective of this study was to assess whether failure of group A streptococcus eradication following treatment for GABHS pharyngitis was associated with specific patient demographic characteristics, specific GABHS M types and colony counts, or viral co-infection.

PATIENTS AND METHODS

Study sites and populations: The Alberta Children's Hospital is a tertiary regional referral centre in Calgary, Alberta that is affiliated with the Faculty of Medicine of the University of Calgary. The hospital serves a population base of 1.2 million people, and children are referred from southern Alberta, southeastern British Columbia and southwestern Saskatchewan. A community-based cohort study was conducted, enrolling patients through five offices of paediatricians and the emergency department of the Alberta Children's Hospital. Children were included in the study if they were between two and 18 years old, complained of sore throat and had one or more of the following physical findings: pharyngeal injection or exudate, temperature higher than 38.4°C or tender cervical lymphadenopathy. Patients were excluded if there was a history of penicillin allergy, antibiotic use in the preceding 72 h, acute rheumatic fever or if informed consent was refused.

Specimens and follow-up: Pharyngeal and nasopharyngeal (NP) swabs were obtained and a prescription was given (penicillin V 50 mg/kg/day tid for 10 days). Physicians could choose to instruct patients to fill the prescription immediately or wait for the throat swab result (rapid test or culture) in 24 to 48 h. Patients positive for GABHS returned for follow-up throat and NP swabs two to five days after the completion of antibiotics. Treatment failure was defined as definite if isolates of the same M and T type were found on initial and follow-up cultures. It was defined as probable if the follow-up swab was positive using the Optical Immunoassay (OIA) (Biostar, United States) but was culture negative. Compliance was defined as consumption of at least 80% of the prescribed doses (1) within 10 days as documented by diary and/or measurement of residual medication. At least one measurement was required for the patient to be evaluated.

Throat swabs were inoculated onto a blood agar plate and incubated anaerobically at 37°C, then inspected at 24 h for beta-hemolytic colonies. The foam plug at the base of the swab transport tube was incubated in Todd-Hewitt broth under the same conditions, subcultured to blood agar plate at 24 h and inspected at 48 h. Those confirmed to be Gram-positive, catalase-negative and bacitracin-sensitive cocci were grouped using the PathDX kit (Intermedico) or Oxoid Diagnostic Reagents (Oxoid Diagnostics). If GABHS grew on agar plates, then bacterial load was determined. Colony counts were categorized (1+ to 4+) according to the number

of colonies in plate quadrants one to four respectively as follows:

- 1+ if less than 10, less than five, zero and zero colonies
- 2+ if greater than 10, greater than five, less than five and zero colonies
- 3+ if greater than 10, greater than 10, greater than five and less than five colonies
- 4+ if greater than 10, greater than 10, greater than five and greater than five colonies

Rapid detection of GABHS by OIA (28,29) was performed after the swab was planted for culture. Serotyping of isolates was performed on the basis of M precipitation and T agglutination (30-32), as well as PFGE with the restriction endonuclease *Sma I* (33) at the National Centre for Streptococcus in Edmonton, Alberta.

The penicillin tolerance of isolates from children with eradication failure was assayed as previously described (34). Briefly, doubling dilutions of penicillin ranging from 8 g/mL to 0.004 g/mL were performed in cation-adjusted Mueller-Hinton broth supplemented with 5% defibrinated horse blood. A 100-fold dilution of a 0.5 McFarland of the test organism was added to each dilution of penicillin and incubated overnight at 37°C. The minimal inhibitory concentration (MIC) was then determined in the last tube with visible growth of the test organism. To determine the minimal bactericidal concentration (MBC), a 10 L aliquot from the first tube showing no growth (the MIC tube) and the four dilutions afterwards were plated and incubated overnight. The antibiotic dilution with 10 colonies or less was considered to be 99.9% kill and was read as the MBC. Tolerance was defined by a fourfold or greater difference in the MBC compared with the MIC.

NP swabs were inoculated onto four cell lines: African green monkey kidney and HEp2 cells at 37°C, and rhesus monkey kidney and human embryonic lung cells at 33°C. Cultures were examined for cytopathic effect at seven and 14 days. Positive results were confirmed using electron microscopy for adenovirus and rhinovirus, direct fluorescent antibody for respiratory syncytial virus, and hemadsorption followed by direct fluorescent antibody testing for influenza A and B, and parainfluenza (35).

Sample size calculation: Sample size calculation was based on the assumptions of the novel hypothesis. The primary outcome measure was the number of children with GABHS microbiological treatment failure in the viral coisolation group compared with those with no virus coisolation. Given the expected frequency of microbiological treatment failure, a fourfold difference in the outcome was considered to have clinical significance. A similar effect has been shown in at least one study of viral coisolation in AOM (25). Therefore, assuming a 20% treatment failure rate in those without viral coisolation and a viral coisolation rate of 10% (19), a total sample of 60 (of whom six had viral coisolation) would provide a power of 0.80 with a two-sided alpha of 0.05. Although GABHS rates as high as almost 40% have been found among children with pharyngitis (19), a conservative estimate of GABHS infection rate (25%) was used to calculate a required, total enrolment sample size of at least 240 children.

TABLE 1
Comparison of characteristics of enrolled patients with group A streptococcus pharyngitis by risk factor

Characteristic	Patients with viral coisolation (n=10) (%)	Patients without viral coisolation (n=94) (%)
Male	5 (50.0)	55 (58.5)
Mean age ± SD (years)	7.4±3.1	7.0±2.5
Symptoms		
Sore throat	9 (90.0)	92 (97.9)
Sore lymph nodes	5 (50.0)	66 (70.2)
Parental report of fever	9 (90.0)	72 (76.6)
Clinical signs		
Inflamed pharynx	9 (90.0)	89 (94.7)
Exudate	4 (40.0)	42 (44.7)
Enlarged and/or tender lymph nodes	6 (60.0)	68 (72.3)
Mean temperature (°C)* ± SD	38.7±0.7	37.7±1.0
Antibiotic started immediately	5 (50.0)	57 (60.6)
Compliant with medication	9 (90.0)	87 (92.6)

*$P<0.01$, t *test with unequal variances*

Ethics: The study protocol was approved by the Research Committee at the Alberta Children's Hospital and the Conjoint Medical Ethics Committee at the University of Calgary.

Data management and statistical analysis: Data were entered into Microsoft Access Version 2.0 (Microsoft Corporation, United States) and analysis was performed using Stata (Stata Corporation, United States). Differences between groups were compared using Fisher's exact test for categorical variables and Student's *t* test for continuous variables. $P<0.05$ was considered statistically significant. Relative risk was calculated with an exact 95% CI.

RESULTS

Study population: A total of 286 children were enrolled between November 1994 and March 1996. Thirty-eight children were excluded from the analysis because of enrolment or protocol violations, or penicillin allergy, leaving 248 children. Twelve of the 38 excluded patients (31.6%) were excluded because of missed follow-up appointments. The majority of enrolments occurred in the first year of the study (195 of 248, 78.6%). GABHS were detected in 104 children (41.9%), nongroup A streptococci were found in six children (2.4%) and 10 children (4.0%) had a virus in the absence of other potential bacterial causes of pharyngitis. All enrolment throat swabs that were positive for GABHS by OIA were also culture-positive by at least one method.

Evaluable patients had a mean age of 7.1 years and were equally divided between the sexes. GABHS-positive patients were more likely to be male (57.7% versus 45.8%, P=0.07), to have tender and/or enlarged lymph nodes (71.2% versus 51.4%, P<0.01) and to be prescribed antibiotics immediately (59.6% versus 29.2%, P<0.001). However, the frequency of viral isolation was similar for those with (10 of 104, 9.6%)

TABLE 2
Patient and laboratory characteristics by bacteriological outcome

Characteristic	Patients with group A streptococcus treatment failure (n=33) (%)	Patients without group A streptococcus treatment failure (n=71) (%)
Demographics		
Entered study in year 1	26 (78.8)	49 (69.0)
Male	21 (63.6)	39 (54.9)
Mean age ± SD (years)*	6.5±2.4	7.3±2.6
Clinical presentation		
Sore throat	32 (97.0)	69 (97.2)
Sore lymph nodes	23 (69.7)	48 (67.6)
Parental report of fever†	22 (66.7)	59 (83.1)
Inflamed throat	30 (90.9)	68 (95.8)
Exudate	16 (48.5)	30 (42.3)
Enlarged and/or tender lymph nodes	25 (75.8)	49 (69.0)
Measured temperature ± SD (°C)‡	37.6±1.0	38.0±1.0
Temperature higher than 38.4°C‡	5 (18.5)	18 (32.7)
Antibiotics started immediately	18 (54.6)	44 (62.0)
Compliant with therapy	30 (90.9)	66 (93.0)

**Fisher exact test, P=0.07; †Fisher exact test, P=0.13. Of the 23 children with no history of fever, six (26.1%) had documented temperature higher than 38.4°C on presentation, eight (34.8%) were afebrile and nine (39.1%) had no temperature recorded; ‡Data recorded for 27 children with treatment failure and 55 with group A streptococcus eradication*

TABLE 3
Group A beta-hemolytic streptococcus (GABHS) M type by bacteriological outcome

M type	Patients with GABHS (n=33) (%)	Patients without GABHS (n=67) (%)	Total
1	5 (15.2)	9 (13.4)	14
2	0	2 (3.0)	2
3	6 (18.2)	15 (22.4)	21
4	4 (12.1)	6 (9.0)	10
6	2 (6.1)	4 (6.0)	6
12*	8 (24.2)	7 (10.4)	15
28	3 (9.1)	10 (14.9)	13
62	0	3 (4.5)	3
77	3 (9.1)	5 (7.5)	8
Nontypeable	2 (6.1)	6 (9.0)	8

**Two-sided Fisher exact test, P=0.08*

TABLE 4
Frequency of colony counts on throat swabs taken at enrolment and follow-up for patients with and without treatment failure

	Patients with GABHS treatment failure (n=29)		Patients without GABHS treatment failure (n=65)*
Colony count	Pretreatment (%)	Post-treatment (%)	Pretreatment (%)
Rare	1 (3.4)	0	3 (4.6)
1+	0	1 (3.4)	3 (4.6)
2+	4 (13.8)	6 (20.7)	5 (7.7)
3+	4 (13.8)	4 (13.8)	8 (12.3)
4+	20 (69.0)	18 (62.1)	46 (70.8)

**χ^2 for trend, P=0.46 for both pretreatment groups. 1+ if less than 10, less than five, zero and zero colonies; 2+ if greater than 10, greater than five, less than five and zero colonies; 3+ if greater than 10, greater than 10, greater than five and less than five colonies; 4+ if greater than 10, greater than 10, greater than five and greater than five colonies. GABHS Group A beta-hemolytic streptococcus*

and without (10 of 144, 6.9%) GABHS-associated pharyngitis. Comparison of GABHS-infected children with and without viral coisolation revealed similar demographic and clinical characteristics (Table 1).

Outcome: Failure to eradicate GABHS occurred in 33 children (31.7%), of whom all but four met the criteria for definite treatment failure. Three of those with probable treatment failure were GABHS-positive at follow-up by OIA (but not culture), and in one case the isolate was lost. One child acquired a new strain of GABHS and was not considered to be a treatment failure. Eleven children (33.3%) with microbiological treatment failure were symptomatic (with any of the same pharyngitis symptoms as at enrolment) at the time of their follow-up visit. Treatment failure occurred in two of 10 children (20.0%) with viral coisolation compared with 31 of 94 (33.0%) without viral coisolation (two-sided Fisher's exact test, P=0.50). The relative

risk of treatment failure with respiratory virus coisolation, compared with no viral coisolation, was 0.57 (95% CI 0.16 to 2.03, Taylor series). Exclusion of the four children with probable treatment failure did not change the results of the analysis (relative risk 0.67, 95% CI 0.19 to 2.40, P=0.72).

Relationship to other variables: There were no statistically significant associations found between the outcome and any other demographic or clinical characteristic. Bacteriological treatment failure was not explained by either early initiation of antibiotics or lack of compliance (Table 2). The latter was analyzed in greater detail by comparing the mean number of doses taken by participants having treatment failure with the mean number taken by those without treatment failure. This was completed using both diary information (28.1±1.6 versus 27.9±2.0, P=0.94) and medication measurement (29.6±1.3 versus 29.9±1.9, P=0.20). However, a trend was found between treatment failure and younger age, as well as a history of fever at presentation (Table 2).

Similarly, there were no microbial factors clearly associated with the outcome. The most common M types overall were M3, M12, M1 and M28, with only M12 being more common among those with treatment failure (24.2% versus 10.2%, P=0.08) (Table 3). Furthermore, there were no significant differences (greater than two bands) within M types using PFGE, nor any differences between pretreatment and post-treatment isolates by PFGE. All but one child with microbiological treatment failure had colony counts of 2+ or greater (Table 4). Penicillin tolerance was not demonstrated in any of the 23 paired pretreatment and post-treatment isolates that were tested.

DISCUSSION

In this study, there were no associations between treatment failure after GABHS pharyngitis and various demographic, clinical and microbial factors. In particular, there was no association between coisolation of a respiratory virus and persistent GABHS detection.

Among the demographic and clinical variables studied, age was associated with treatment failure. Although it did not reach statistical significance, the children with treatment failure in our study were, on average, one year younger than those who had GABHS eradicated. Carriers under the age of 15 years have previously been shown to have a higher rate of microbiological treatment failure compared with older patients (36). A recent retrospective study over a 20-year period showed recurrences to be significantly more common in younger children (aged one to eight years) than in older children (aged 13 to 19 years) (37). These findings are consistent with another study which show a reduced local pharyngeal immune response to GABHS in younger children compared with older children (38). If this immune response is important in the eradication of GABHS in the pharynx, younger children may be more likely to have GABHS treatment failures. None of the other demographic and clinical factors appeared to have predictive use for GABHS eradication.

Of the microbial factors studied, only M type 12 showed a trend toward association with the outcome. An outbreak of M12 pharyngitis was previously noted to be associated with high penicillin treatment failure rates (10), although in this study, M12 isolates comprised only 15% of the enrolled GABHS-infected children. We questioned whether there could be a strain-associated characteristic that predisposed patients infected with this M type to treatment failure. However, there are no studies of endemic GABHS pharyngitis that have investigated the relationship between treatment failure and specific M type, so this relationship cannot be corroborated. M12 is a common cause of uncomplicated streptococcal pharyngitis (39) but has been associated with post-streptococcal glomerulonephritis (40) and invasive infection (41). It is possible that there are strain-specific characteristics that impair its clearance from the pharynx; however, this possibility requires further investigation.

It is intriguing that, while respiratory viruses appear to have no relationship to bacterial persistence in GABHS pharyngitis, they do seem to play such a role in AOM. Because the viral effects on respiratory epithelial cells seem to be similar, anatomy may account for the outcome differences. While continued mucosal swelling secondary to the viral infection may not have an impact in the pharynx, it may delay the restoration of patency of the eustachian tube and, therefore, the drainage of middle ear fluid and its bacterial pathogens (27).

Conducting this study over two years ensured a variety of viral isolates and GABHS serotypes. Including more than one viral season reduced the likelihood of the effect of predominant viruses having very strong or weak correlations with GABHS treatment failure. Enrolment over several seasons also allowed us to assess the potential role of multiple GABHS serotypes. Although the majority of enrolments occurred in the first year of the study, this was not associated with outcome (Table 2).

NP swabs were chosen for viral detection because they are technically easier to perform and are better tolerated (42). False-negative viral cultures were unlikely, given the prompt delivery of specimens and the similarity of viral isolation rates to past studies (6.9% in the GABHS-negative group). Positive viral cultures were found in 10.2% in one paediatric pharyngitis study (43) and 15.7% in another (19). Enrolment criteria may explain slight differences in viral frequency between these studies. If false-negative viral cultures did occur, their frequency should not have differed between those with and without GABHS eradication and, therefore, would not have altered the results.

The high treatment failure rate in this study (33.7%) necessitates the examination of other potential explanations. Poor compliance (12) and penicillin tolerance (13) have been linked to microbiological treatment failure, but neither explains the treatment failure rates in this study. Chronic carriage has been suggested to comprise up to 20% of treatment failures (44) and has been distinguished from acute infections by the lack of a serological response (45). Antistreptolysin O testing was not performed given both ethical and practical concerns about performing nonbeneficial blood tests in children. Moreover, the correlation of serum immune response to true infection status has been questioned (46,47) and, therefore, may not be a fail-safe means of identifying carriers. The lack

of difference in virus isolation between patients in whom GABHS was and was not eradicated suggests that chronic GABHS carriage alone is unlikely to account for the treatment failures seen in the present study. Similar treatment failure rates have been noted in other study populations (48).

The absence of a suitable explanation for the GABHS treatment failure rate in this study suggests that other theories should be explored. Previous investigators have proposed that BLPF and/or GABHS-inhibitory pharyngeal organisms (not cultured in this study) may play a role in this phenomenon (16,49). BLPF is postulated to break down penicillin in vivo, thus preventing its action against GABHS and resulting in treatment failure (49). Thus, broader spectrum agents have been promoted to overcome this problem (3). Normal oral flora such as alpha-streptococci have been shown to be antagonistic to GABHS (50), preventing colonization (51) and reducing carriage and infection (16,49). This theory is still considered controversial. Alternatively, it is possible that other factors, such as penicillin penetration into pharyngeal or tonsillar tissue and host factors, also play a role in treatment failure.

High treatment failure rates, particularly when associated with symptoms, have prompted some authorities to suggest a need to re-examine penicillin as the first treatment choice in such populations (48,52). Some physicians have noted that patient dissatisfaction was higher in cases of symptomatic than asymptomatic treatment failure (local pediatricians, personal communication). However, given the concern about the use of broad spectrum antibiotics and the lack of evidence that acute rheumatic fever outbreaks are due to decreased penicillin efficacy (53), most experts continue to favour penicillin as the drug of choice (53-55).

CONCLUSIONS

The present study did not demonstrate an association between any demographic, clinical or microbial factors, including that of viral coisolation and bacterial treatment failure after GABHS pharyngitis. However, the contributory roles of M type and age warrant further evaluation.

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