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Biofilm Formation by Group A Streptococci: Is There a Relationship with Treatment Failure?

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Group A streptococcus (GAS) is the primary cause of bacterial pharyngitis in children and adults. Up to one-third of patients treated for GAS pharyngitis fail to respond to antibiotic therapy. The objective of this cohort study was to evaluate GAS biofilm formation as a mechanism for antibiotic treatment failure using previously collected GAS isolates and penicillin treatment outcome data. The minimum biofilm eradication concentration (MBEC) assay device was used to determine the biofilm-forming capabilities, efficiencies, and antibiotic susceptibilities of GAS isolates. The MBECs and MICs of several antibiotics for GAS were determined. All 99 GAS isolates available for this study formed biofilms, with various efficiencies. Antibiotic MBECs were consistently higher than MICs for all of the GAS isolates. MBECs indicated penicillin insensitivity in 60% of GAS isolates, producing the first report of in vitro GAS insensitivity to penicillin. Using MBECs to predict penicillin treatment failure had better sensitivity (56%) but lower specificity (36%) than the sensitivity (0%) and specificity (100%) when MICs were used. However, the positive predictive value of the MBEC was superior to that of the MIC (56 versus 0%), while the negative predictive values (42 and 47%) were similar. More studies are needed to understand the roles of biofilms and the MBEC assay in predicting GAS treatment failure. In addition, further investigations are necessary to determine if non-biofilm-forming strains of GAS exist and the roles of in vivo monospecies and multispecies biofilms in streptococcal pharyngitis treatment failure.

Group A streptococcus (GAS) commonly causes acute pharyngeal infections in children 2 to 18 years of age, irrespective of gender and often during the colder months of the year (24, 25). Infections are normally self-limited. However, antibiotic treatment relieves discomfort, minimizes transmission, and reduces the occurrence of complications (5).

Microbiologic treatment failure is the inability of antibiotics to eradicate pharyngeal GAS following a full course of therapy. Although no strain of GAS that is resistant to penicillin in vitro has been identified in clinical laboratories using current susceptibility methods, treatment failure rates of up to one-third have been observed in clinical practice (5, 16, 20). Nonetheless, penicillin remains the antibiotic of choice when treating GAS infections because of its efficacy, safety, narrow spectrum of effect, and low cost (5). Mechanisms that may explain penicillin treatment failure have been explored extensively. These mechanisms include elimination of commensal organisms (6, 13, 28), viral copathogenicity (16), internalization of GAS in epithelial cells (21, 23), and bacterial copathogenicity (6, 7, 28, 34). To date, none of these mechanisms, either individually or in combination, completely explains the occurrence of penicillin treatment failure observed in clinical practice. Given that GAS has been shown to form biofilms (15), evaluation of GAS biofilm formation as a comprehensive theory for the occurrence of GAS treatment failure with commonly used antibiotic therapies (penicillin, macrolides, cephalosporins, and clindamycin) is justified.

Numerous studies have demonstrated that biofilms are inherently less susceptible to a variety of antibiotic therapies than the same bacteria grown in planktonic form (9, 17, 22). These findings hold true for gram-negative and gram-positive microorganisms, including species of streptococci, with a diverse range of antibiotic types (9, 22). At present, antibiotic susceptibility is measured by standards set out by the National Committee for Clinical Laboratory Standards (NCCLS) in which the MIC of an antibiotic is determined for bacteria in planktonic form only. This study evaluated biofilm formation as a potentially important mechanism contributing to GAS treatment failure across all types of antibiotic therapies, using the minimum biofilm eradication concentration (MBEC) to determine the antibiotic susceptibility of GAS in the biofilm form. The objective was to determine if GAS isolates capable of biofilm formation would be more common in penicillin treatment failure and if MBECs indicating insensitivity would correlate with penicillin treatment outcome.

MATERIALS AND METHODS

Parent study. The GAS isolates used for biofilm testing were obtained during a previous prospective community cohort study, hereafter referred to as the parent study, evaluating the role of viral copathogenicity in penicillin treatment failure (16). The parent study consisted of children 2 to 18 years of age who presented at five offices of pediatricians in Calgary, Canada, and the emergency department of the Alberta Children's Hospital, Calgary, experiencing symptoms associated with pharyngeal infection between November 1994 and March 1996.

Study subjects. Children presenting with sore throats and one or more physical symptoms (pharyngeal injection or exudate, temperature of >38.4°C, or tender cervical lymphadenopathy) were included in the study if they agreed to participate. Patients were excluded if they had a history of penicillin allergy or acute rheumatic fever or antibiotic use in the preceding 72 h or if enrollment or the study protocol was violated.

Parent study design. After pharyngeal swabs were obtained for GAS culture, each child was prescribed penicillin V at 50 mg/kg of body weight/day for 10 days. Patients whose swabs came back positive for GAS returned 2 to 5 days following

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the completion of penicillin treatment to undergo a second throat swab. Compliance with the recommended treatment was monitored by diary entry and audit of residual medication. Consumption of 80% of the recommended dosage within 10 days was considered sufficient compliance.

Definition of outcome variables. Penicillin treatment success was defined as the inability to identify GAS from the pharyngeal swab taken during the follow-up physician visit. Penicillin treatment failure was defined as (i) definite if isolates of the same M and T types were found on initial and follow-up cultures and (ii) probable if the follow-up swab was optical immunoassay positive but culture negative.

Biofilm formation. GAS isolates and outcomes of GAS penicillin treatment failure or success were available from the parent study (16). Clinical isolates were obtained from throat swabs collected during the first physician visit of the parent study. The distribution of M types was as follows: M3, 21%; M12, 15%; M1, 14%; M28, 13%; M4, 10%; M77 and nontypeable, 8% each; M6, 6%; M62, 3%; and M2, 2%. There was no difference in penicillin treatment outcome by M type (16). Isolates were inoculated in glycerol solution and frozen at −70°C for the duration of storage; no passaging was done.

The laboratory analysis of GAS isolates was performed using the MBEC assay device developed in the Biofilm Laboratory, University of Calgary (9). The laboratory protocol has been described in detail in an earlier publication (9). GAS isolates frozen at −70°C were recovered on 5% sheep blood agar and incubated for 24 h at 35°C. Biofilm-forming capability was measured by the presence of cell growth after the sonicated bacteria from the MBEC assay device were spot plated (9). Sterility controls were run using random interspacing of broth wells with each MBEC plate. The number of bacteria involved in biofilm formation was determined after an incubation period of 24 h by direct count of CFU, representative of viable-cell counts. The CFU for each GAS biofilm were evaluated in two separate trials. The two measurements were within a factor of 10 for 81 (82%) of the 99 GAS isolates. Isolates with CFU measurements that differed by more than a factor of 10 were reevaluated in a third trial. CFU were reported as an average of all the trials completed. The continuous distribution of log CFU was divided into tertiles labeled low ($\leq 10^3$), medium ($>10^3$ but $\leq 10^4$), and high ($>10^4$) efficiencies of biofilm formation. Scanning electron micrographs (SEM) of MBEC assay pegs were performed on two isolates from each tertile to ensure that efficiencies of $<10^2$ were still representative of biofilm formation.

Antibiotic susceptibility testing. Antibiotic susceptibility testing of a subset of the GAS isolates capable of forming biofilms was reported as MICs and MBECs. Five GAS isolates were randomly selected from the penicillin treatment failure and success groups in each classification of efficiency (low, medium, and high) in forming biofilms following the first efficiency trial ($n = 30$). Random selection was achieved by separating slips of paper with each GAS isolate code into six groups (penicillin treatment failure with low, medium, or high efficiency in forming a biofilm and penicillin treatment success with low, medium, or high efficiency in forming a biofilm) and blindly choosing five slips from each group. Five GAS isolates from each group were chosen in order to achieve a balance among time, cost, and obtaining the data necessary to meet study objectives. All the selected isolates from failures were from definite treatment failures; 6 involved symptomatic (at follow-up) treatment failures, while 10 involved asymptomatic treatment failures. During the selection processes, one treatment failure was misclassified as a success and an uneven number of successes ($n = 14$) and failures ($n = 16$) were tested. There were no demographic or symptomatic differences between the patients in the total sample and those whose isolates were selected for further study (data not shown).

The antibiotics used in susceptibility testing of the GAS isolates were as follows: penicillin, erythromycin, clindamycin, cephalexin, ceftriaxone, rifampin, and a combination of penicillin and rifampin. The MBEC assay device trough system was used to produce seven equal biofilms of a single GAS isolate for antibiotic susceptibility testing. NCCLS standard growth medium for antibiotic susceptibility was used: Mueller-Hinton broth and 2% lysed horse blood.

Planktonic antibiotic susceptibility testing involved the measurement of the MIC of each antibiotic for the subset of GAS isolates that were successful in forming biofilms. The planktonic MIC was obtained using an MBEC assay; this assay has been validated against the NCCLS (9). Biofilm antibiotic susceptibility testing involved the measurement of the MBEC of each antibiotic for the same subset of GAS isolates successful in forming biofilms.

The concentrations of antibiotics used for MIC and MBEC measurement were <0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and >512 μg/ml. Resistance was predicted for each GAS isolate if the MIC exceeded the peak concentration in serum, and insensitivity was predicted if the MBEC exceeded the peak concentration in serum. The peak concentrations in serum for the antibiotics were as follows: penicillin, 5 μg/ml; penicillin plus rifampin, 7 μg/ml; rifampin, 7 μg/ml; erythromycin, 2 μg/ml; clindamycin, 10 μg/ml; cephalexin, 18 μg/ml; and ceftriaxone, 270 μg/ml. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of using MBECs and MICs to anticipate the outcome of penicillin treatment failure and success were calculated. We felt that clinically, a positive result from the point of view of prediction of treatment outcome would be the ability of the test to identify those patients for whom therapy is likely to fail. Thus, PPV in this context refers to the ability of MBEC or MIC results to predict those patients for whom therapy is likely to fail.

Data analysis. Data were entered into Microsoft Access version 3.0 and converted to a Stata-compatible form. All calculations were done using the statistical program Stata version 7.0. Descriptive analysis included frequency, mean, and range where applicable. Binomial proportion comparisons, Student *t* tests, two-sided Fisher's exact tests, and chi-square tests for trends were used in the analysis (29).

Ethical approval. Ethical approval for the study was obtained through the University of Calgary Conjoint Medical Ethics Committee on 3 May 2001.

TABLE 1. Penicillin treatment success and failure

Variable	Penicillin treatment/outcome[b]		*P* value
	Failure ($n = 32$ [32%])	Success ($n = 67$ [68%])	
Age (yr)			
≥2, <5	8 (25)	13 (19)	
≥5, <9	20 (62)	38 (57)	
≥9, <16	4 (13)	16 (24)	0.22[c]
Gender, male	21 (66)	35 (52)	0.21
1st visit symptoms			
Sore throat	31 (97)	65 (97)	0.97
Sore glands	23 (72)	48 (72)	0.98
Fever	21 (66)	55 (82)	0.07
Inflamed pharynx	29 (91)	63 (94)	0.54
Pharyngeal exudates	14 (44)	29 (43)	0.97
Enlarged glands	25 (78)	45 (67)	0.26
Biofilm formation	32 (100)	67 (100)	1.00
Biofilm log CFU			
Mean	3.3 (SD = 1.0)	3.2 (SD = 1.0)	0.55
Low ($\leq 10^3$)	13 (41)	30 (45)	
Medium ($> 10^3$, $\leq 10^4$)	13 (41)	26 (39)	
High ($> 10^4$)	6 (18)	11 (16)	0.68[d]

[a] By age, gender, symptoms at the first physician visit, biofilm formation, and efficiency of biofilm formation among children 2 to 18 years of age in Calgary, Alberta, Canada, from November 1994 to March 1996.
[b] Number (percent). SD, standard deviation.
[c] *P* value for the relationship between age and penicillin treatment outcome.
[d] *P* value for the relationship between efficiency of biofilm formation and penicillin treatment outcome.

RESULTS

A total of 248 patients met the evaluation criteria for determination of GAS infection in the parent study (16). Of these subjects, 104 had positive GAS swabs on their first physician visit. The GAS isolates for five of these subjects were not available for biofilm testing and were excluded from further analysis, leaving 99 isolates.

The sample ($n = 99$) was divided into penicillin treatment failures ($n = 32$) and successes ($n = 67$), based on data from the parent study. The ages and gender distributions of the patients in the treatment success and failure groups were similar (Table 1). The proportions of subjects presenting with sore throat, sore glands, fever, inflamed pharynx, pharyngeal exudates, and enlarged glands on the first physician visit were equivalent in the two groups (Table 1). There was no differ-

TABLE 2. GAS isolates for which MICs indicate resistance and MBECs indicate insensitivity by biofilm-forming efficiency

Antibiotic (dose)	No. of isolates (%)								*P* value for insensitivity in relation to increasing efficiency
	MIC				MBEC				
	L	M	H	Total (n = 50)	L	M	H	Total (n = 30)	
Penicillin (5 μg/ml)	0	0	0	0 (0.0)	6	5	7	18 (60)	0.65
Penicillin + rifampin (7 μg/ml)	0	0	1	1 (0.3)	9	9	10	28 (93)	0.38
Rifampin (7 μg/ml)	1	1	5	7 (23)	7	9	10	26 (87)	0.05
Erythromycin (2 μg/ml)	0	0	0	0 (0.0)	9	9	10	28 (93)	0.38
Clindamycin (10 μg/ml)	0	0	0	0 (0.0)	8	9	10	27 (90)	0.14
Cephalexin (18 μg/ml)	0	0	0	0 (0.0)	9	8	9	26 (87)	1.00
Ceftriaxone (270 μg/ml)	0	0	0	0 (0.0)	8	8	6	22 (73.3)	0.32

[a] L, low-efficiency biofilm formation ($\leq 10^3$); M, medium-efficiency biofilm formation ($>10^3$, $\leq 10^4$); H, high-efficiency biofilm formation ($>10^4$).

ence in the risk of treatment failure based on biofilm formation, as all GAS isolates (n = 99) formed biofilms, whether they belonged to the penicillin treatment failure (100%) or success (100%) group.

SEM showed that GAS was able to attach, organize, and begin biofilm formation even at the low CFU (10^1 to 10^2 CFU) efficiency. These rudimentary biofilms had minimal exopolysaccharide matrix, but they were clustered at the air-fluid interphase of the MBEC assay device peg and were not diffusely spread over the peg surface. Beyond 10^2, SEM showed that biofilm clustering and complexity is more obvious (photographs are available).

The efficiencies of biofilm formation, measured in log CFU, were similar in the GAS isolates from patients with penicillin treatment failure and those with success (P = 0.55) (Table 1). The variables age, gender, and symptoms at the first physician visit were analyzed by efficiency in forming biofilms, but none of these variables were associated with increasing efficiency of biofilm formation (data not shown).

The penicillin MICs for none of the GAS isolates indicated resistance; however, the MBECs for 18 of 30 (60%) GAS isolates indicated insensitivity to penicillin. The MIC for one GAS isolate showed resistance to the combined penicillin-rifampin regimen. In contrast, the MBECs for only two GAS isolates were considered to indicate sensitivity to the penicillin-rifampin antibiotic regimen (Table 2).

All GAS biofilms were insensitive to at least one of the antibiotics tested, and 12 (40%) GAS isolates were insensitive to all of the antibiotics tested. No relationship was found between increasing efficiency of biofilm formation and antibiotic insensitivity (Table 2).

Using MBEC-predicted insensitivity to penicillin to anticipate penicillin treatment failure had a sensitivity of 56.25% and a specificity of 35.71% (Table 3). The MICs predicted that there would be no penicillin treatment failures, resulting in a lower sensitivity (0%) but a specificity of 100% (Table 4). The PPV of the MBEC was superior to that of the MIC (56 versus 0%), while the NPVs (42 and 47%) were similar. The sensitivity and PPV of using the MBEC for screening may have been slightly inflated due to the imbalance of GAS isolates in the categories of success (n = 14) and failure (n = 16). However, the measurements of the MIC screening tool were not affected, given that the MIC was unable to identify failures.

DISCUSSION

Biofilm formation alone did not explain penicillin treatment failure in our patient sample. GAS isolates from both the treatment failure and success groups were able to form biofilms, and some of these biofilms were sensitive to penicillin. Therefore, the capability to form a biofilm alone does not confer penicillin insensitivity and subsequent penicillin treatment failure. Findings similar to these, including reports involving other streptococcal species, have indicated that not every biofilm has a lack of sensitivity to antibiotics (22).

Biofilms have a variety of attributes that contribute synergistically to the process of antibiotic insensitivity. These attributes include, but are not limited to, a lower growth rate (30, 32), an exopolysaccharide matrix (12, 18), a change in gene expression (3, 11, 26), an optimal three-dimensional structure (1, 8, 35), and the production of potentially resistant genes (2). GAS may form a biofilm, but it requires one or more of these attributes to be present within the biofilm in order to facilitate antibiotic insensitivity.

The efficiency of GAS biofilm formation after 24 h, measured in log CFU, was normally distributed with a range of 10^1 to 10^5. Other gram-positive and streptococcal organisms have been shown to grow 24-h biofilms with total cell numbers near

TABLE 3. MBEC exceeding peak concentration of penicillin in serum and clinical penicillin treatment outcome[a]

MBEC prediction	Treatment outcome		Total
	Failure	Success	
Failure	9	9	18
Success	7	5	12
Total	16	14	30

[a] *P* value, 0.65; sensitivity, 56.25% (9 of 16); PPV, 50.00% (9 of 18); specificity, 35.71% (5 of 14); NPV, 41.67% (5 of 12).

TABLE 4. MIC exceeding peak concentration of penicillin in serum and clinical penicillin treatment outcome[a]

MIC prediction	Treatment outcome		Total
	Failure	Success	
Failure	0	0	0
Success	16	14	30
Total	16	14	30

[a] *P* value, 1.00; sensitivity, 0.0% (0 of 0); PPV, 0.0% (0 of 0); specificity, 100.0% (14 of 14); NPV, 46.67% (14 of 30).

10^4 or 10^5 (22, 30, 32). However, no other studies have looked at a variety of isolates of the same species. This finding indicated that there was a spectrum of capacities for GAS isolates to initiate or expand the biofilm. Factors that contribute to the initiation and expansion of biofilms are numerous; some examples include the capability to adhere to the substratum (12), quorum sensing (19), growth rate (32), and establishing a biofilm structure (30, 35). Using the total number of cells in the biofilm to measure efficiency was insufficient to establish the factor(s) that would assist in explaining the variation observed in the efficiency of GAS biofilm formation. Thus, additional factors that contribute to the process of constructing a GAS biofilm warrant further exploration.

Increasing efficiency of biofilm formation did not correlate with patient symptoms, penicillin treatment failure or success, or antibiotic insensitivity. Although these findings are important because they eliminate a marker that may have been used clinically to predict symptomatology, virulence, or antibiotic susceptibility, they are not entirely surprising because the number of cells composing a biofilm is not one of the attributes that have been shown to contribute to biofilm success.

GAS biofilms were consistently more insensitive to antibiotic therapy than their planktonic forms, similar to other biofilm-forming bacteria, including a variety of streptococcal species (9, 22). In fact, GAS biofilms demonstrated some degree of lack of sensitivity to all antibiotic classes tested, and all GAS strains were insensitive to at least one of the antibiotics tested. In this study, there were no GAS isolates for which the MIC of penicillin indicated resistance. This is consistent with previous studies that have not been able to identify any strains of GAS that are resistant to penicillin in laboratory analysis of planktonic growth forms, even though in the clinical setting, failure occurs in up to one-third of patients. A similar phenomenon occurs with *Pseudomonas aeruginosa* (27). Biofilm formation is the first mechanism able to show that GAS can be insensitive to traditional penicillin therapy, so like biofilm formation in *P. aeruginosa*, it may more accurately reflect what is occurring in patients.

We chose to compare the MIC with the MBEC because the former is the routine clinical measurement used in susceptibility testing for GAS and previous reports have shown that the MICs of penicillin for all GAS isolates indicate that they are susceptible to this antibiotic. We were exploring the possibility that MBECs are more representative of susceptibility and would perhaps be more useful in the clinical setting. The sensitivity and specificity of using laboratory MBEC data to predict penicillin treatment failure are likely too poor for current clinical use. Whereas the NPVs are similar, the MBEC PPV is a major improvement over that of MICs in anticipating penicillin treatment failure. There are two aspects of the finding that MBECs were unable to accurately predict penicillin treatment failure that require some additional discussion.

First, penicillin treatment failure occurred in the clinical setting when the MBEC for the GAS isolate would have predicted susceptibility. This could be attributed to biofilm susceptibility testing being conducted on an immature biofilm. If the 24-h incubation was not sufficient for the GAS isolate to reach its maximum potential (growth plateau), then the results of the susceptibility testing would not accurately reflect what will occur with the mature biofilm within the host. There are also environmental factors that may affect GAS antibiotic susceptibility in the host. Infection with copathogenic organisms is a theory that has been explored previously with GAS and organisms such as *Staphylococcus aureus*, *Haemophilus parainfluenzae*, and *Moraxella catarrhalis*, which produce beta-lactamase, showing mixed results. Biofilms may also become multispecies as they develop, incorporating other species of bacteria in the biofilm in order to create a mutually beneficial relationship for survival. Such a biofilm attribute is frequently inherent in organisms causing infectious disease (1). A multispecies biofilm containing *Streptococcus pneumoniae* and *M. catarrhalis* was found to protect pneumococci from concentrations of penicillin and amoxicillin that would have indicated insensitivity (8). Patients may have developed multispecies biofilms with copathogenic organisms that added to the biofilm attributes, causing penicillin insensitivity when insensitivity was not expected based on the laboratory MBECs.

Second, penicillin treatment success occurred in the clinical setting when the MBEC for the GAS isolate would have predicted a lack of sensitivity. In this case, additional host factors or environmental factors may have stopped biofilm formation from occurring. The effect of a host's immune response on the formation of a biofilm can occur throughout the stages of biofilm growth (4, 31). A recent study found that lactoferrin, a factor in innate immunity which is a ubiquitous component of human excretions, stops *P. aeruginosa* from adhering and initiating biofilm formation (31). In the host environment, there are commensal organisms playing a role in disrupting the GAS infection as well. This is another theory that has been explored in current GAS literature with some success, but not in the context of biofilms. Alpha hemolytic streptococcus, a bacterium that contributes to the host's normal flora, can compete or interfere with GAS growth. Many aspects of this theory may apply to biofilms and could be revisited in the context of biofilm formation. Commensal organisms may impede the formation of a biofilm by disrupting the biofilm structure or intercepting quorum-sensing signals, reducing biofilm growth. With either or both of these mechanisms, a commensal organism may make GAS susceptible to antibiotic therapy when laboratory MBEC data predict insensitivity.

The antibiotic that was found to be most successful in eradicating GAS biofilms in vitro was penicillin (40%), followed by cephalosporins (ceftriaxone, 27%; cephalexin, 13%), rifampin (13%), clindamycin (10%), erythromycin (7%), and penicillin plus rifampin (7%). Finding that penicillin was the antibiotic most likely to be effective in eradicating biofilms was surprising, since this antibiotic has the highest treatment failure rate in clinical studies. It has also been shown to be one of the least effective antibiotics for biofilm eradication with other organisms, and a mechanism for its inability to act on biofilms has been described in the literature (1). However, insensitivity of 60% of GAS isolates is significant. This suggests that a variety of host and environmental factors must be involved for penicillin to be as successful as it is in clinical practice.

It was also curious that ceftriaxone and other cephalosporins appeared to be less effective than penicillin in the laboratory but have been found to be more effective clinically (1). In addition to all the host and environmental factors affecting biofilm formation in vivo, which cannot be captured in vitro, cephalosporins have the added advantage that they are not

impeded by beta-lactamase production and are not as efficient in eliminating some commensal organisms.

Clindamycin, erythromycin, and rifampin were all less effective than we would have anticipated given what is observed in clinical practice. The mechanisms of activity for these three antibiotics generally require a bacterial cell to be metabolically active in order for the drug to be effective. Biofilm cells have significant changes in gene regulation and show a slower growth pattern; therefore, they may have a natural reduction in the production of RNA and proteins. These antibiotics may require a longer duration of exposure in order to be effective.

The identification of one GAS isolate that was resistant to penicillin plus rifampin was interesting. However, the combination of beta-lactam antibiotics and rifampin has previously been shown to result in an antagonistic relationship in some bacteria, where the bactericidal activity of the beta-lactam is decreased by the presence of rifampin (10, 36). The antagonistic relationship of this antibiotic combination was reinforced further by the biofilm data. In at least 11 GAS isolates, the MBEC for penicillin-rifampin therapy indicated insensitivity when the same GAS isolate was sensitive to either penicillin or rifampin alone.

The inclusion of carriers in the patient sample represents a potential limitation of this study. Carriers harbor GAS organisms in their noses and throats without experiencing symptoms of acute infection or expressing evidence of an immune response (33). Often children who are carriers are eliminated from treatment failure studies because their infections do not elicit a serologic response and are therefore different from most GAS infections. Patients who may have been carriers were not excluded during the parent study, since it was deemed to be unethical and impractical to draw blood from a child without a therapeutic purpose (16). However, it is unlikely that this decision had an appreciable impact on the present study results. Every GAS isolate was identified as susceptible to penicillin therapy when grown in planktonic form prior to treatment. Therefore, based on current clinical laboratory standards for testing antibiotic susceptibility, no penicillin treatment failures were expected. It is possible that carriage may also be explained by the formation of a mature biofilm. Biofilms are consistent with carriage in that they protect bacterial cells from antibiotic therapy and impede the host immune system from recognizing the presence of infection (14). Although this study cannot address the issue of carriage directly, there was no advantage in excluding carriers for the purpose of the study.

In the future, investigations are needed to determine if non-biofilm-forming strains of GAS exist and to examine what role they may play in the pathogenesis of GAS infection. In addition, further exploration of the role that biofilms and the MBEC assay device play in predicting GAS treatment failure is warranted. Studies may also be conducted to determine the part of in vivo monospecies and multispecies biofilms in streptococcal pharyngitis treatment failure.

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