

## ERYTHROMYCIN-RESISTANT GROUP A STREPTOCOCCI IN SCHOOLCHILDREN IN PITTSBURGH

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### ABSTRACT

**Background** Resistance to erythromycin has been very uncommon among group A streptococci in the United States.

**Methods** As part of a longitudinal study, we obtained surveillance throat cultures twice monthly and with each new respiratory tract illness from children in kindergarten through grade 8 at one school in Pittsburgh. Screening for resistance to erythromycin and clindamycin was initially accomplished with use of the Kirby-Bauer disk-diffusion test. The minimal inhibitory concentration of resistant isolates was determined by the E test. A double disk-diffusion test was used to characterize the resistance phenotype, and the polymerase-chain-reaction assay was used to identify the resistance gene. The molecular relatedness of strains was determined by field-inversion gel electrophoresis.

**Results** A total of 1794 throat cultures were obtained from 100 children between October 2000 and May 2001, of which 318 cultures (18 percent) from 60 of the children were positive for group A streptococci. Forty-eight percent of these isolates (153 of 318) were resistant to erythromycin. None were resistant to clindamycin. Results of the double disk-diffusion test indicated the presence of the M phenotype of erythromycin resistance. Molecular typing indicated that the outbreak was due to a single strain of group A streptococci. Of 100 randomly selected isolates of group A streptococci obtained from the community between April and June 2001, 38 were resistant to erythromycin.

**Conclusions** In January 2001, during a longitudinal study of schoolchildren, we detected the emergence of erythromycin resistance in pharyngeal isolates of group A streptococci. This clonal outbreak also affected the wider community. (N Engl J Med 2002;346:1200-6.)

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**G**ROUP A streptococci are the most frequent and important cause of bacterial pharyngitis in children and adults.<sup>1,2</sup> Although many antibiotics are effective for the treatment of streptococcal pharyngitis, penicillin V remains the drug of choice.<sup>3,4</sup> Erythromycin is recommended for persons who are allergic to penicillin.<sup>3,4</sup> Azithromycin is not recommended as first-line therapy for pharyngitis due to group A streptococci; however, many practitioners find the five-day regimen of

one dose of azithromycin per day attractive. Azithromycin and other macrolide antibiotics are also frequently prescribed for nonstreptococcal pharyngitis and other upper respiratory tract infections, and the rates of prescription of azithromycin have increased.<sup>5-7</sup> The increased use of macrolide antibiotics is correlated with an increased rate of resistance to erythromycin among isolates of group A streptococci.<sup>8,9</sup>

As part of an ongoing longitudinal study, we examined the antimicrobial-susceptibility patterns of pharyngeal isolates of group A streptococci from schoolchildren and evaluated the sudden emergence and rapid spread of erythromycin resistance among these isolates in January 2001.

### METHODS

#### Study Setting and Subjects

In 1998 we began a longitudinal study of the epidemiology of infections with group A streptococci in a private, tuition-supported elementary school with an enrollment of approximately 285 children. This school serves children of the faculty of the University of Pittsburgh, is located in Pittsburgh, and includes kindergarten through grade 8. The children are divided into three major groups according to age: 5 to 7 years, 8 to 10 years, and 11 to 13 years. Each group has multiple classrooms, and most classes have 12 to 15 students. All classrooms are represented in the study. There is considerable daily contact among children within a group.

All students attending the school were invited to join the study, and all students who were willing to participate were enrolled. The protocol was approved by the institutional review board of Children's Hospital of Pittsburgh. Written informed consent was obtained from the parents of the children who participated, and assent was also obtained from the children.

The initial study cohort comprised 48 children between the ages of 5 and 13 years. In year 2 of the study, 75 children were followed, and in year 3 of the study, 100 children were followed. Forty-one of the original cohort have remained in the study for all three years. The majority of the children were followed for more than one year. Forty-seven percent of the students are girls, 74 percent are white, 10 percent are black, and 16 percent are another or of mixed racial background. The demographic characteristics of the children in each study year were similar to those of the school as a whole.

#### Processing of Throat Cultures

Throat swabs were obtained for culture approximately every two weeks from each child during each school year from October through May (17 sets of cultures per school year) and each time a child had a new respiratory tract illness. Follow-up cultures were

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obtained two to four days after the completion of antibiotic therapy for an acute episode of pharyngitis due to group A streptococci to determine whether there was colonization.

Throat cultures were obtained by swabbing both tonsils and the posterior pharynx with a rayon-tipped swab (BBL Becton Dickinson, Sparks, Md.). The swab was then placed in Amies medium without charcoal, transported to the laboratory, and plated within two hours. The swab was plated onto 5 percent sheep's-blood agar; the agar was stabbed, and a bacitracin disk was placed in the primary streak (BBL Becton Dickinson). The plates were incubated at 37°C in 5 percent carbon dioxide and were examined at 24 and 48 hours.  $\beta$ -Hemolytic colonies were subcultured, isolated, and then typed (Pathodx, Diagnostic Products, Los Angeles). All throat swabs were obtained by one of two investigators; the processing of throat cultures was overseen by one senior technician.

### Clonality of the Outbreak

The genetic relatedness of erythromycin-resistant strains was investigated by field-inversion gel electrophoresis, a type of pulsed-field gel electrophoresis, according to previously described methods.<sup>10</sup> A 4-mm slice of each sample was initially digested with 24 U of *Sma*I in 1× restriction buffer (New England Biolabs, Beverly, Mass.) and held at room temperature for at least 16 hours. Similar digestions were carried out with *Apa*I, *Eag*I, or both in their respective restriction buffers at appropriate temperatures.

The migration of DNA bands was compared with that of the lambda-ladder standard. Bands falling between 50 and 250 kb were used for the analysis. The bands were compared visually, and the degree of clonal relatedness was interpreted according to guidelines proposed by Tenover et al.<sup>11</sup> Isolates were considered to be indistinguishable from one another if the patterns of bands on field-inversion gel electrophoresis matched. Isolates were categorized as closely related if the difference between patterns was no more than three bands. Isolates were considered to be possibly related if the patterns differed by four to six bands. Isolates were considered to be different if the patterns differed by at least seven bands. One or more representative isolates of each pattern identified during the first two years of the study and four erythromycin-resistant strains from the third year were selected and sent to the Centers for Disease Control and Prevention for *emm* typing (i.e., comparison of the sequence of the 5' *emm* variable region in a strain with the sequence of a known, or reference, strain).<sup>12</sup> The *emm* gene encodes the M protein, which governs the ability of group A streptococci to resist phagocytosis.

### Susceptibility Testing

Isolates of group A streptococci collected as part of the longitudinal study were initially screened for their susceptibility to erythromycin and clindamycin with use of the Kirby-Bauer disk-diffusion test (BBL Becton Dickinson) on Mueller-Hinton agar with 5 percent sheep's blood according to published guidelines.<sup>13</sup> The minimal inhibitory concentration (MIC) was then determined with use of the E test (AB Biodisk, Piscataway, N.J.) for isolates that either had an intermediate level of susceptibility or were resistant to erythromycin or clindamycin. Susceptibility to erythromycin as well as to clindamycin was defined by a MIC of no more than 0.25  $\mu$ g per milliliter, an intermediate level of susceptibility was defined by a MIC of 0.5  $\mu$ g per milliliter, and resistance to erythromycin was defined by a MIC of at least 1.0  $\mu$ g per milliliter.<sup>13</sup> The isolates were stored at -70°C for later use.

### Mechanism of Resistance

To identify the mechanism of resistance, we used a double disk-diffusion test.<sup>14</sup> Mueller-Hinton agar plates supplemented with 5 percent sheep's blood were inoculated with a suspension of group A streptococci that met a McFarland 0.5 turbidity standard. An erythromycin disk (concentration, 15  $\mu$ g per milliliter) and a clin-

damycin disk (concentration, 2  $\mu$ g per milliliter) were placed 16 mm apart (edge to edge) on each plate. Resistance to erythromycin, with blunting of the zone of inhibition around the clindamycin disk on the side of the erythromycin disk, indicates an inducible MLS<sub>B</sub> phenotype (resistance to most macrolide, lincosamide, and streptogramin B antibiotics); resistance to both erythromycin and clindamycin indicates a constitutive MLS<sub>B</sub> phenotype; and susceptibility to clindamycin, with no blunting of the zone around the erythromycin disk, indicates an M phenotype (resistance to macrolide antibiotics). The M phenotype is associated with the *mefA* gene, and the MLS phenotypes are associated with the *erm* gene.

We used a polymerase-chain-reaction (PCR) assay to identify the genetic mechanism of resistance of eight representative isolates of the macrolide-resistant *emm* 6 clone from eight children. The *mefA*, *ermB*, and *ermTR* resistance genes were detected by PCR amplification with the use of previously described primers.<sup>15</sup> Several isolates of *Streptococcus pyogenes* that were susceptible to erythromycin and were obtained during year 3 of the study were used as negative controls. The expected sizes of the PCR products were 616, 348, and 206 bp for *ermB*, *mefA*, and *ermTR*, respectively.

### Erythromycin-Resistant Group A Streptococci in the Community

After the presence of erythromycin-resistant isolates of group A streptococci was noted in the elementary school, we determined the prevalence of such isolates in the surrounding community. In April, May, and June of 2001, we randomly selected 100 isolates of group A streptococci from pharyngeal cultures (which had been stripped of identifying information) that had been processed in the clinical microbiology laboratory of the Children's Hospital of Pittsburgh. These cultures were from children who were seen at the primary care clinic (40 percent) or the emergency department (60 percent) of the Children's Hospital of Pittsburgh. The largest source was socioeconomically diverse patients who sought emergency care after hours and on weekends. Susceptibility testing was performed as described for the isolates obtained from the children in the surveillance study.

### Clinical Definitions

A child was classified as a carrier if group A streptococci were recovered from at least two sequential surveillance cultures obtained two weeks apart in the absence of symptoms. A child was categorized as having recurrent infections if positive cultures, indicating clinical or subclinical infections with group A streptococci, were separated by a minimum of two negative cultures during a three-week period in which the child was not receiving antibiotic therapy. A child was designated as having had a single episode of infection if a single positive throat culture was preceded and followed by negative throat cultures. Infections were classified as clinical or subclinical; clinical infections were further categorized as typical or atypical. Typical infections were those in which sore throat was a prominent symptom. Atypical infections were those in which rhinorrhea without sore throat predominated. Subclinical infections were those in which the child had no symptoms.

### Treatment

Patients with new symptoms of a respiratory tract illness (rhinorrhea with or without sore throat) and a new infection with group A streptococci were treated with penicillin V or amoxicillin by a single study physician. At the beginning of the longitudinal study, erythromycin was prescribed only for children who were allergic to penicillin. Starting in February 2001, clindamycin was used for patients who were allergic to penicillin. Children without symptoms of a respiratory tract illness were not treated with an antibiotic. Symptoms were assessed when the throat cultures were obtained; each time a positive culture for group A streptococci was obtained, the parents were called to inform them of the results and to inquire about further symptoms.

## RESULTS

## Prevalence of Macrolide-Resistant Isolates

A total of 2200 throat cultures were obtained during the first two years of the study (October 1998 to May 2000); 322 throat cultures (15 percent) were positive for group A streptococci.<sup>16,17</sup> All isolates were sensitive to erythromycin. During the third year of the study (October 2000 to May 2001), 1794 cultures were obtained, of which 318 isolates (18 percent) from a total of 60 children were positive for group A streptococci. Of these 318 isolates, 153 (48 percent) were resistant to erythromycin (the MIC that inhibited the growth of 50 percent of the isolates was 32  $\mu\text{g}$  per milliliter, and the MIC that inhibited the growth of 90 percent of the isolates was 32  $\mu\text{g}$  per milliliter) by the E test. All the isolates were sensitive to clindamycin. At least one erythromycin-resistant isolate was recovered from 46 of the 60 children (77 percent) who were infected during the 2000–2001 school year (range, 1 to 10). The first erythromycin-resistant isolate was recovered in January 2001; resistant isolates continued to be obtained throughout the remainder of year 3 of the study. Figure 1 shows the prevalence of erythromycin-sensitive and erythromycin-resistant isolates of group A streptococci during each of the three years of the study.

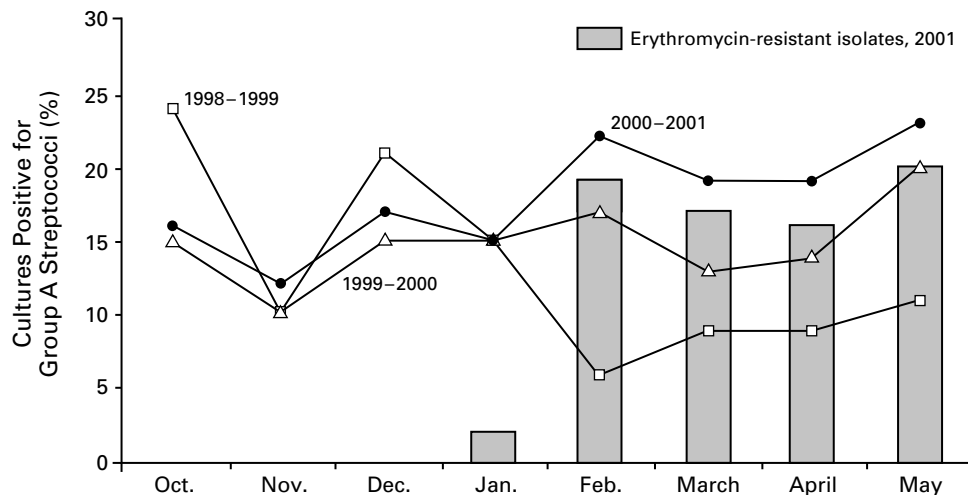
## Clinical Features

Table 1 shows the types of infections and treatment of the 46 children from whom macrolide-resistant group A streptococci were recovered. Twenty-two of the 46 children (48 percent) had a single positive

culture. Two children (4 percent) had recurrent episodes of infection with erythromycin-resistant group A streptococci. Twenty-two children (48 percent) had multiple serial cultures that were positive for group A streptococci, and they were considered to be carriers. The mean duration of carriage within this subgroup of children was 11.5 weeks (range, 3 to 18). Children with symptomatic infections with erythromycin-resistant isolates were indistinguishable from children with symptomatic infections with macrolide-susceptible group A streptococci. All children with symptomatic infections were treated with penicillin, amoxicillin, or clindamycin; consequently, the efficacy of erythromycin or other macrolide agents in the treatment of pharyngitis due to erythromycin-resistant group A streptococci could not be determined.

## Clonality of the Outbreak

Field-inversion gel electrophoresis was performed on all isolates of group A streptococci from each of the 46 children. Because field-inversion gel electrophoresis of the first 14 isolates did not yield interpretable banding patterns with the use of the restriction endonuclease *Sma*I, we subsequently used both *Eag*I and *Apa*I as restriction endonucleases. Digestion with *Apa*I produced 14 bands, whereas the use of *Eag*I produced 13 bands. The pattern on field-inversion gel electrophoresis was identical in the case of 44 of the 46 erythromycin-resistant isolates (Fig. 2). Two isolates, which each differed by one band, were categorized as closely related. These results indicate that the erythromycin resistance seen in this outbreak was due to a single clone.



**Figure 1.** Percentage of Throat Cultures Positive for Group A Streptococci during Each Year of the Study, 1998–2001. Each year represents an academic year (i.e., October through May). Each data point represents the number of positive cultures divided by the total number of cultures obtained. The bars depict the number of erythromycin-resistant isolates as a percentage of the total number of cultures obtained.

**TABLE 1.** CHARACTERISTICS OF THE FIRST EPISODE OF A GROUP A STREPTOCOCCAL INFECTION AND TREATMENT WITH A MACROLIDE-RESISTANT ISOLATE.

TYPE OF INFECTION AND TREATMENT*	NO. OF CHILDREN (%)
Typical	23 (50)
Amoxicillin	12†
Penicillin	10†
Clindamycin	1
Atypical	5 (11)
Amoxicillin	4
Penicillin	0
Clindamycin	1
Subclinical	18 (39)
Amoxicillin	0
Penicillin	0
Clindamycin	0

\*Typical infections were those in which a sore throat was the predominant symptom. Atypical infections were those in which rhinorrhea without sore throat predominated. Subclinical infections were those in which a child had no symptoms.

†Two patients began treatment with a macrolide antibiotic; within 48 hours, once sensitivity data were available, treatment was changed to amoxicillin or penicillin and the course of therapy was completed.

Four representative isolates of resistant group A streptococci from year 3 of the study were selected and submitted to the Centers for Disease Control and Prevention for *emm* testing. The isolates were determined to be *emm* 6. In the first two years of the study, 23 of the 322 isolates of group A streptococci (7 percent) were *emm* 6.<sup>16,17</sup>

#### Mechanism of Resistance

We used the double disk-diffusion test to identify the mechanism of resistance of all isolates of group A streptococci recovered from each of the 46 children. None of the isolates caused blunting of the zone of inhibition around the clindamycin disk, indicating that all had the M phenotype of erythromycin resistance. Results of the PCR assay indicated the presence of a single, 348-bp band consistent with the presence of the *mefA* gene in all eight macrolide-resistant *emm* 6 isolates that were tested. These bands were not observed in the macrolide-susceptible isolates of group A streptococci (Fig. 3).

#### Community Isolates

We selected 100 pharyngeal isolates of group A streptococci at random from the clinical laboratory between April and June 2001. Susceptibility testing showed that 38 of the isolates (38 percent) were resistant to erythromycin. Neither field-inversion gel electrophoresis nor *emm* typing was performed on these isolates.

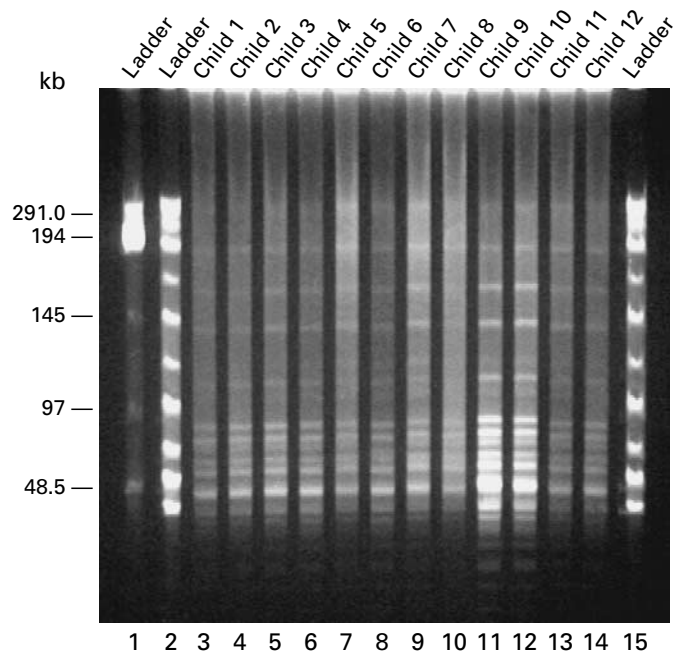
#### DISCUSSION

Beginning in the mid-1980s, the incidence of acute rheumatic fever increased and serious and invasive diseases caused by group A streptococci emerged while the incidence of pharyngitis due to group A streptococci remained stable.<sup>18,19</sup> Although the reasons for these changes are unclear, the fact that they occurred emphasizes the need to identify children with streptococcal pharyngitis correctly. Prompt initiation of appropriate and effective antibiotic therapy will prevent suppurative and some nonsuppurative complications of infections with group A streptococci. It will also reduce the pool of patients from which adults and other children acquire their infections.

The susceptibility of group A streptococci to commonly prescribed antibiotics has been very stable in the United States.<sup>20-22</sup> In a recent survey of 301 isolates from 24 states and the District of Columbia, all were sensitive to penicillin, ceftriaxone, and clindamycin, and only 2.6 percent were resistant to erythromycin and azithromycin.<sup>21</sup> However, many other countries, including Japan,<sup>23</sup> Korea,<sup>24</sup> Spain,<sup>25,26</sup> Italy,<sup>27,28</sup> Finland,<sup>29,30</sup> and Greece,<sup>31</sup> have a high prevalence of isolates that are resistant to erythromycin and other macrolide antibiotics. In Finland, the rates of erythromycin resistance among group A streptococci prompted an intense effort to decrease the use of macrolide antibiotics, which in turn led to a dramatic reduction in the prevalence of erythromycin-resistant strains.<sup>30</sup> Similarly, a marked decline in the incidence of erythromycin-resistant isolates in Japan followed a steep decrease in the use of macrolide antibiotics.<sup>23</sup> The rates of resistance that we observed in Pittsburgh are similar to those reported in Europe and Japan.

We found an unexpectedly high incidence of erythromycin-resistant isolates of group A streptococci among children in a single school in the United States. A parallel, though less systematic, surveillance for erythromycin resistance in the isolates of group A streptococci from the clinical laboratory of the Children's Hospital of Pittsburgh showed a high prevalence of such isolates also in the community. Pulsed-field gel electrophoresis is the most sensitive and standard tool used to investigate the epidemiologic characteristics of an outbreak of infections with group A streptococci in a community.<sup>32-34</sup> We used field-inversion gel electrophoresis, a form of pulsed-field gel electrophoresis, to show that this outbreak was due to a single clone of group A streptococci. The appearance of this clone may represent the introduction of a resistant strain of *emm* 6 into this school and community; alternatively, the resistance gene may have been acquired by a strain of *emm* 6 that was already present in the population.

The increasing use of macrolide antibiotics has



**Figure 2.** Results of Field-Inversion Gel Electrophoresis of Erythromycin-Resistant Isolates of Group A Streptococci.

The endonuclease *EagI* was used for digestion. Lane 1 shows the lambda-ladder concatemers from *Saccharomyces cerevisiae* DNA, lanes 2 and 15 show the mid-range lambda ladder, and lanes 3 through 14 show erythromycin-resistant isolates of group A streptococci from 12 children.

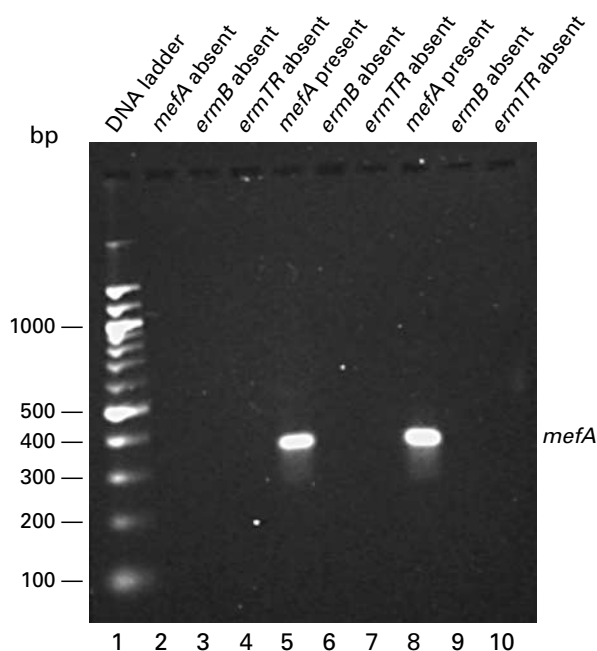
been documented in several studies in the United States.<sup>5-7</sup> This trend has been accelerated by wide use of short courses of azithromycin for the treatment of pharyngitis, otitis media, sinusitis, and community-acquired pneumonia in both children and adults. The children in our study did not routinely receive macrolide antibiotics for their acute infections with group A streptococci. Review of local pharmacy data from the largest insurer in our area showed that the use of macrolide antibiotics increased steadily from July 1998 through June 2001.

The children in our study were only treated for group A streptococci pharyngitis if they had respiratory symptoms and a new positive throat culture for group A streptococci. The children with erythromycin-resistant isolates were all treated with amoxicillin, penicillin, or clindamycin as directed by the study protocol. The outcome of the treatment of pharyngitis due to a streptococcal isolate that is resistant to macrolide antibiotics is uncertain. The rate of failure may be higher than expected.<sup>35</sup> The frequency of treatment failure in children infected with erythromycin-resistant group A streptococci has varied; in Finland, the rate was 47 percent,<sup>29</sup> whereas in Italy it was approximately 40 percent.<sup>27,36</sup>

The emergence of erythromycin resistance among pharyngeal isolates of group A streptococci might easily remain unnoticed, given the widespread replacement of conventional cultures with methods of rapid antigen detection for diagnosis. Even when throat cultures are obtained, susceptibility testing is not routinely performed. Furthermore, children with streptococcal pharyngitis usually recover within several days, even in the absence of treatment with appropriate antibiotics, and the performance of a routine "test of cure" is not recommended.<sup>3,4</sup> Only in the event of an increased frequency of suppurative, invasive, or nonsuppurative complications of infection with erythromycin-resistant group A streptococci might this trend toward erythromycin resistance be detected.

A careful history obtained from the family of the first student identified as having macrolide-resistant group A streptococci failed to identify either recent travel or visitors as a risk factor. However, many of the children in this school are not participating in our study; accordingly, the index patient may not have been one of the children who have been followed longitudinally. Regardless of the source, the isolate spread very rapidly within the school population.

It is essential to determine whether the high prev-



**Figure 3.** Results of Polymerase-Chain-Reaction Assay of Group A Streptococci to Identify Erythromycin-Sensitive Strains (Lanes 2, 3, and 4) and Erythromycin-Resistant Strains (Lanes 5 through 10) That Were Positive for the *mefA*, *ermB*, and *ermTR* Resistance Genes.

Lane 1 shows the DNA ladder. The erythromycin-sensitive isolates were negative for *mefA* (lane 2), *ermB* (lane 3), and *ermTR* (lane 4). The erythromycin-resistant isolates were positive for *mefA* (lanes 5 and 8) and negative for *ermB* (lanes 6 and 9) and *ermTR* (lanes 7 and 10).

absence of erythromycin-resistant group A streptococci is confined to our region or is widespread. We recommend that macrolide antibiotics not be used for the routine treatment of pharyngitis due to group A streptococci until more epidemiologic information is available or unless susceptibility testing is first performed.

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