Short Communication

Correspondence
Thangam Menon
thangam16@rediffmail.com

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Biotypes of group A streptococci isolated from children

M. Palani Kumar, 1 Thangam Menon, 1 Charmaine Lobo, 1 N. Anbumani, 1 C. P. Girish Kumar 1 and S. Shanmugasundaram 2

1Department of Microbiology, Dr. ALM PGIBMS, University of Madras, Taramani, Chennai – 600 113, India
2Department of Cardiology, Stanley Medical College, Chennai – 600 001, India

Thirty-eight isolates of group A streptococci from patients with pharyngitis, 13 isolates from patients with pyoderma and 28 carrier strains were subjected to biotyping by carbohydrate fermentation tests and production of β-glucuronidase. Biotype 10 was observed most frequently among clinical isolates and biotypes 3 and 4 were most common among carrier isolates.

Introduction

Group A streptococci (GAS) are important human pathogens that cause acute pyogenic infections, such as pharyngitis and pyoderma. They are also responsible for non-suppurative sequelae, such as rheumatic fever, acute glomerulonephritis and rheumatic heart disease (Bisno, 1991).

Molecular methods that are currently used for typing GAS are expensive, time-consuming and difficult to interpret. There is therefore a need for more reliable, economical and simple methods of GAS strain differentiation. Commercially available identification systems have been used by previous workers for strain biotyping (Bouvet et al., 1994). There are, however, no reports on GAS biotypes in India. This is a preliminary report of GAS biotypes that are seen in patients with pharyngitis or pyoderma and healthy carriers in a south Indian paediatric population.

Methods

Bacterial isolates. The study included 38 GAS isolates from cases of pharyngitis, 13 isolates from cases of pyoderma and 28 isolates from throat swabs of healthy children. All subjects were 5–15 years of age. Specimens were transported to the laboratory by using the filter paper strip method (Johnson et al., 1997). The filter paper strips were incubated on the surface of tryptose blood agar plates overnight at 37 °C under 5–7 % CO2. β-Haemolytic GAS were identified by colony morphology and grouping of strains was done by using a Streptex kit (Abbott Murex). Isolates were stored at −70 °C in Robertson’s cooked meat medium with 10 % glycerol until they were processed.

Biotyping. GAS isolates were biotyped by their ability to ferment mannitol, cyclodextrin, glycogen, methyl β-D-glucopyranoside and pullulan and the production of β-glucuronidase (Bouvet et al., 1994). Sugar fermentation reactions were performed by using serum peptone water that contained 1 % sugar. Tubes were inoculated with strains of GAS and readings were taken after overnight incubation. Glucuronidase activity was detected by impregnating a disc of Whatman filter paper (No. 1) with the substrate (4-methylumbelliferyl β-D-glucuronide) and incubating with bacterial culture for 30 min at 37 °C, after which it was examined for fluorescence under UV light.

Results

Results were interpreted by comparison with the standard reactions described by Bouvet et al. (1994) (Table 1). Biotyping performed for GAS delineated seven different biotypes among throat isolates, three different biotypes among pus isolates and eight different biotypes among carrier isolates.

Of the 38 GAS strains that were isolated from children with pharyngitis, 13 GAS strains were of biotype 10 and seven strains were of biotype 4. Biotypes 2, 3, 5, 8 and 9 were encountered less frequently.

Of the 13 GAS strains that were isolated from cases of pyoderma, eight strains belonged to biotype 10. Biotypes 1, 6 and 7 were not seen in any of the clinical cases.

Of the 28 GAS strains that were isolated from healthy children, six strains belonged to biotype 4 and five strains were of biotype 3. Biotypes 1, 2, 5, 6, 8 and 9 were less common (Table 2). Biotypes 7 and 10 were not present among carrier strains.

Biotype 10 was observed most frequently among clinical isolates and biotypes 3 and 4 were the most common among carrier isolates. Biotype 10, which was observed predominantly in clinical strains, was not observed in carrier strains.

Discussion

The association of specific biotypes with clinical disease suggests that there may be a pathogenic association of some
biotypic characteristics with virulence factors. In a survey of 1–14-year-old children in Paris, biotypes 1–6 were most common in the pharyngitis cohort; hence, there appear to be striking epidemiological differences in the overall frequencies of biotypes in different geographical regions (Bouvet et al., 1994).

Table 1. Standard biotype reactions of GAS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result by type</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidification of:</td>
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<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mannitol</td>
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<td>+</td>
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<tr>
<td>Cycloextrin</td>
<td></td>
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<td></td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glycogen</td>
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<tr>
<td>Pullulan</td>
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<td>+</td>
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<tr>
<td>Methyl β-D-glucopyranoside</td>
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<td></td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Production of β-glucuronidase</td>
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<td></td>
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</tr>
</tbody>
</table>

Clear-cut individualization of biotypes was found to be easy and reproducible, with one enzymic test (β-glucuronidase) and carbohydrate fermentation tests leading to constant and unambiguous results (Table 2).

Biotyping appears to be a simple method of characterizing strains of GAS, particularly in laboratories where molecular characterization may not be possible.

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References

