Molecular Epidemiology of Nosocomial Rotavirus Infection

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ABSTRACT

The molecular epidemiology of nosocomial rotavirus infection in children admitted to the paediatric ward of a large hospital in southern India was studied by transmission electron microscopy and RNA electrophoresis on stool samples. Six hundred and twenty-six samples were collected and rotaviruses were detected in 35 of them. A predominant electropherotype was found in all the samples, and there was no seasonal variation in the incidence of infection. The predominant electropherotype pattern in this study was different from the electropherotype patterns of strains isolated elsewhere in the country and also from the pattern of the strains isolated earlier from children with acute diarrhoea at the same hospital.

Key words: Rotavirus; Diarrhoea, Infantile; Gastroenteritis; Epidemiologic methods; Rotavirus infections; Cross infections

INTRODUCTION

Rotavirus-associated gastroenteritis is a major public health problem of infants and young children. Rotaviruses share common antigens, and human strains are difficult to cultivate, that alternative approaches are necessary to study the epidemiology of rotavirus infection in humans. One of these methods is to study the migration patterns of RNA segments of rotaviruses separated by electrophoresis on polyacrylamide gels, which is both characteristic and constant for a given strain. Electropherotyping has proven to be a useful method in studying genomic variations, tracing double infections, characterizing the number of strains in outbreaks and showing the considerable genome diversity in human rotaviruses (1-9).

We report here the molecular epidemiology of nosocomial rotavirus infection in children admitted to the Paediatric Ward of the Christian Medical College and Hospital, Vellore, South India, from November 1990 to September 1991.

MATERIAL AND METHODS

Faecal samples

This study was carried out in 194 children aged less than three years admitted to the paediatric ward without any enteric symptoms on admission or without any history of diarrhoea during the week prior to admission. A total of 626 faecal samples were collected from the children on the third day of admission (to exclude any infections acquired before admission), and then every third day till discharge. The children were closely monitored, and if they developed diarrhoea (increase in stool frequency in conjunction with excessive water loss) a stool sample was collected immediately, after 2 days and then every third day till discharge. At discharge, the parents of the children were requested to bring the children immediately to hospital if the children developed diarrhoea within three days of discharge. Thirty-nine of the children developed nosocomial gastrointestinal infection during the study.

RNA extraction and electrophoresis

Extraction of viral RNA was carried out as previously described (8). Briefly, a 10% suspension of stool on phosphate-buffered saline was homogenized, and clarified
by low-speed centrifugation. The supernatant containing virus particles was treated with sodium dodecyl sulphate, and then deproteinized with a phenol-chloroform mixture. The RNA was precipitated with a sodium acetate and ethanol mixture, pelleted by centrifugation and resuspended in sample buffer for electrophoresis. Electrophoresis was carried out at 10 mA for 18 hours at room temperature in 7.5% polyacrylamide gel using the discontinuous system described by Laemmli (10) without sodium dodecyl sulphate. The gels were stained using a modification of the method described by Herring et al. (11). After fixation in 40% ethanol containing 10% acetic acid for 30 minutes and then in 10% ethanol with 0.5% acetic acid for a further 30 minutes, the gel was soaked in an 11 mM silver nitrate solution for 30 minutes, washed in distilled water and reduced in a solution of 0.75 M sodium hydroxide containing 0.3% formaldehyde for 4-5 minutes. The reaction was stopped with 5% acetic acid. The migration pattern was classified according to the scheme proposed by Lourenco et al. (6) as modified by Dimitrov et al. (12).

### Electron microscopy

All stool samples from the patients included in the study were subjected to an electron microscopic examination for enteric viruses as described earlier (13).

### Bacterial culture

The stool samples were simultaneously cultured on blood agar, MacConkey agar, Xylose Lysine Deoxycholate agar, Deoxycholate citrate agar and Butzler’s agar to identify any bacterial enteropathogens present along with the viruses.

### RESULTS

Viral agents were detected by electron microscopy in 64 samples collected from 28 of the 39 children with nosocomial gastrointestinal infection. Electron microscopic analysis and RNA electropherotyping of all the samples included in the study showed that rotavirus was the most common viral agent detected in 35 of the samples collected from 13 children with nosocomial gastrointestinal infection. The infection was asymptomatic in eight children, whereas other five had mild diarrhoea. The other viral agents detected by electron microscopy were: adenoviruses, calciviruses, enteroviruses, small round viruses and coronavirus-like particles (Table I). Coronavirus-like particles were morphologically similar to coronaviruses, but they could not be cultured and identified with the known serotypes of coronaviruses (14). After detection, rotaviruses were shed in the stool of the children for the next three days. Rotavirus was detected throughout the study period (Table II).

The rotaviral RNA from all except one of the positive samples had the electropherotype Ie, IIb, IIIk, and IVc. The rotaviral RNA from one sample had the electropherotype Ie, IIb, IIIg, IVc. (Fig. I). This sample was received toward the end of the study in August 1991.

Bacterial enteropathogens were also detected in five samples. *Campylobacter* species was grown from a stool sample that had coronavirus-like particles. In four other samples that had rotaviruses, one of the following bacteria was also grown from each sample: *Salmonella typhimurium*, enteropathogenic *Escherichia coli* O:55, *Campylobacter* species, and enteropathogenic *E. coli* O26:B6.

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**Table I. Isolates**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus electropherotype</td>
<td>34</td>
</tr>
<tr>
<td>Ie, IIb, IIIk, IVc</td>
<td></td>
</tr>
<tr>
<td>Rotavirus electropherotype</td>
<td>1</td>
</tr>
<tr>
<td>Ie, IIb, IIIg, IVc</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>11</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>8</td>
</tr>
<tr>
<td>Small round virus</td>
<td>2</td>
</tr>
<tr>
<td>Coronavirus-like particles</td>
<td>7</td>
</tr>
<tr>
<td>Calicivirus</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table II. Samples with rotavirus**

<table>
<thead>
<tr>
<th>Month</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. November 1990</td>
<td>2</td>
</tr>
<tr>
<td>2. December 1990</td>
<td>5</td>
</tr>
<tr>
<td>4. February 1991</td>
<td>6</td>
</tr>
<tr>
<td>5. March 1991</td>
<td>2</td>
</tr>
<tr>
<td>6. April 1991</td>
<td>5</td>
</tr>
<tr>
<td>7. May 1991</td>
<td>5</td>
</tr>
<tr>
<td>8. June 1991</td>
<td>1</td>
</tr>
<tr>
<td>9. July 1991</td>
<td>1</td>
</tr>
<tr>
<td>10. August 1991</td>
<td>4</td>
</tr>
<tr>
<td>11. September 1991</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. Column a and b show the predominant RNA electropherotype Ie, IIb, IIIk, IVc; column c shows the RNA electropherotype Ie, IIb, IIIg, IVc obtained from a single sample.
DISCUSSION

Thirty-nine of the 194 children developed nosocomial gastrointestinal infection, and rotaviruses of a single electropherotype were identified as the commonest viral pathogen. Hospital-acquired rotaviral gastrointestinal infection would, thus, appear to be an important health problem in this age group.

Nosocomial rotavirus infection was detected throughout the study period without observing any characteristic seasonal pattern. However, there were reports on rotavirus-associated diarrhoea where a definite seasonal pattern has been observed (15-20). In an earlier study on children with rotavirus-associated diarrhoea (17), we have reported two peaks of rotavirus infection—one between December and February, and the other between June and August. However, in the neonatal nursery at Vellore, no seasonality was found in nursery-acquired and apparently asymptomatic rotavirus infection (21). It appears likely that factors determining seasonality of rotavirus infection in the community may be different from those in hospital-acquired infection.

The predominant electropherotype pattern in this study is different from the electropherotype patterns of the strains isolated elsewhere in the country (18-20) and earlier at Vellore (17). Between August 1983 and July 1985 at Vellore (17), the prevalent electropherotypes in the community-acquired infection were: Ie, IIb, IIIg, and IVc, whereas the predominant electropherotype patterns for the present nosocomial infection are: Ie, IIb, IIIk, and IVc.

In earlier reports on nosocomial transmission of rotavirus infection (22,23), a minimum of at least 10 different circulating electropherotypes were detected other than in outbreaks due to totally new strains (24,25), as in nosocomial transmission of community strains introduced in the special care units (26). The presence of one predominant circulating electropherotype, as found in this study, is rather unusual, although the second electropherotype identified toward the end of the study may indicate a possible change in the pattern of transmission. It is possible that the predominant strain that was found to propagate in the ward was more resistant to environmental factors, was more pathogenic, and/or had an antigenic structure that was new to the hospital environment. Further studies on the antigenic structure, the nucleotide sequences in the genes coding for the major rotaviral antigens and their association with increased pathogenicity of strain and/or resistance to environmental factors are required to substantiate this assumption.

The presence of children with rotavirus infection in the same ward seems to be of prime importance in the transmission of infection. Since most children in this study were in diapers, the diapers may have been a source of infection. Whether adults transferred viruses to other children (by changing their diapers, bathing them, etc.), or whether direct child-to-child contact or fomites play a role in the transmission is not clear. A respiratory mode of transmission has been proposed, but it has not been substantiated yet (27).

Since nosocomial gastrointestinal infection can prolong hospital stay and increase morbidity of the affected patients, it is necessary to take steps to prevent its occurrence. Care in the handling and proper disposal of the faecal material, in addition to proper hand-washing techniques, would go a long way in the prevention of nosocomial rotavirus infection. There are a number of candidate vaccines presently undergoing clinical trials (28). It may, therefore, soon be possible to immunize high-risk children against rotavirus infection during their hospital stay.

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REFERENCES


