Diarrhea due to non-typhoidal Salmonella refers to the disease caused by serotypes of the genus Salmonella other than Salmonella enterica serotype Typhi. The association of different Salmonella serogroups with acute diarrhea has been reported from various parts of the world, including India (1-6). S. enterica serotype Worthington has been reported as a causative agent of acute diarrhea, particularly in children (7). The aims of the present study were to determine the antimicrobial resistance profiles of S. enterica serotype Worthington strains isolated from fecal samples of children with acute diarrhea and to determine the clonal relationships among those strains.

A total of 68 S. enterica serotype Worthington isolates from the feces of children suffering from acute diarrhea who were admitted to the B. C. Roy Children’s Hospital, Kolkata, India, were included in this study. The isolation and biochemical identification of the organisms were carried out by standard laboratory methods and confirmed by serogrouping with slide agglutination using specific grouping antisera. All the isolates with presumptive identification of Salmonella spp. were finally identified at the National Salmonella Centre, Central Research Institute, Kasauli, India.

The Kirby-Bauer disk diffusion method was used following Clinical and Laboratory Standards Institute guidelines (formerly NCCLS) (8). The antibiotic discs were purchased from Difco (Detroit, Mich., USA). The antibiotics tested were ciprofloxacin, ofloxacin, azithromycin, ampicillin, ceftriaxone, gentamicin, chloramphenicol and co-trimoxazole. Quality control was performed by using Escherichia coli ATCC 25922 as a reference strain.

Plasmid DNA was isolated by using a rapid alkaline lysis method (9).

Pulsed-field gel electrophoresis (PFGE) was performed by clamped homogeneous electric field (CHEF) electrophoresis on a CHEF-DR III system (Bio-Rad Laboratories, Richmond, Calif., USA) according to a standard procedure (10).

The strains were screened for the integrons by polymerase chain reaction (PCR) using three sets of primers specific for the intI1, intI2, and intI3 genes coding for the integrase (11).

Transmission of non-typhoidal Salmonella to humans occurs by ingestion of contaminated food and water as well as by contact with infected animals or contaminated medical instruments. The antimicrobial resistance profiles of the 32 isolates are depicted in Table 1. Overall, 62.5% of strains exhibited resistance to more than three of the eight antimicrobial agents tested. A high percentage of isolates were resistant to azithromycin, ampicillin, gentamicin and ceftriaxone. The appearance of 20 isolates of S. enterica serotype Worthington resistant to ceftriaxone in this study poses a serious therapeutic problem. As reported earlier, the plasmid mediated transferable resistance to third-generation cephalosporins (12), rendering possible the transmission of these resistant genes to other strains of Salmonella. In this study, the most common resistance profiles were azithromycin, ampicillin, ceftriaxone and gentamicin (47%). Multi-drug-resistant non-typhoidal Salmonella has been reported from various parts of the world. Mahajan et al. (13) reported similar findings from Mumbai, India. In the United States, the number of Salmonella organisms that were resistant to one or more antimicrobials rose significantly from 16% in the 1980s to 31% in the 1990s (14). A similar situation has been found in the United Kingdom (15). Moreover, non-typhoidal Salmonella resistant to the newly introduced antimicrobial agents has also been reported (16). Ghadge and Bal (17) reported that S. enterica serotype Worthington isolates, isolated from cases of meningitis and septicemia in neonates, were resistant to chloramphenicol (86%). However, we observed that the majority of the S. enterica serotype Worthington strains were susceptible to chloramphenicol and fluoroquinolone. This is in accordance with the report by Yang et al. from Taiwan (18). Antibiotic-resistant genes are often identified in plasmids, transposons and integrons. The majority of the S. enterica serotype Worthington strains harbored 3.9 kb plasmid. A total of 68 strains of S. enterica serotype Worthington were examined for the presence of integrons. Amongst these, 5 (7.4%) strains were positive for class 1 and only 1 strain was positive for class 2 integron. These data indicate that class 1 integron indeed contributed to the emergence of multi-drug-resistant strains, and that the analysis of integron may be a

Table 1. Antimicrobial resistance profile of Salmonella enterica serotype Worthington isolates (n = 32)

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>No. of resistant strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>21 (65)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>20 (62.5)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>21 (65.6)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>28 (87.5)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>4 (12.5)</td>
</tr>
</tbody>
</table>

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useful molecular epidemiological tool. The majority of the virulence genes are encoded in Salmonella pathogenicity islands, which also encode type III secretion systems used to deliver translocated effector proteins to host cells. PFGE of XbaI-digested genomic DNA revealed that the strains of S. enterica serotype Worthington in this study were heterogeneous in their clonal relationships (Fig. 1). This indicates that multiple clones are in circulation. PFGE typing is an important molecular technique for epidemiological typing of many bacterial pathogens. The usefulness of this technique for typing different serotypes of Salmonella has been reported (19). The presence of multiple clones suggests that infection originated from different sources.

REFERENCES


