induced mice paw oedema, whereas the others did not show any noticeable activity. The percentage of anti-inflammatory activity was in the range of 0–33.9 for different title compounds (at the dose of 1/5 of their ALD\textsubscript{50}) as compared to 49.6\% shown by indomethacin (at the dose of 10 mg/kg).

The data in Table 2 for anti-inflammatory activity show that only three compounds (1, 9 and 12) have some activity (28.3, 24.6 and 33.9\% respectively). The highest activity was observed for the compound with N-p-tolyl-piperazino methyl group at position-3 of the parent nucleus. Moreover, compounds, which are grossly CNS stimulant, are inactive in their anti-inflammatory activity.

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COLONIZATION FACTOR ANTIGENS AND SEROGROUPS OF ENTEROTOXIGENIC ESCHERICHIA COLI

RADHIKA MIRCHANDANI K. D. SHARMA, D. S. CHITNIS AND R. S. KAMAT
Department of Clinical Pathology, Haffkine Institute, Parel, Bombay 400 012, India.

ABSTRACT

309 strains of E. coli isolated from 274 cases of sporadic diarrhoea were studied. 61.2\% of these were enterotoxigenic E. coli (ETEC). 67\% of the ETEC were CFA negative and 58.7\% showed no haemagglutinating pili. The isolates were serogrouped for O-antigens. The predominant serogroups encountered amongst ETEC were 01, 02, 07, 017, 011 and 060. These appear to be different from those reported in the literature. The predominant serogroups amongst normal colonic E. coli were also the same. Isolation of a large number of strains of ETEC without CFA, indicates that the role of CFA in the adhesion of ETEC is over rated. Comparable high frequencies of a few serogroups amongst ETEC and non-toxigenic E. coli is highly significant from the point of view of epidemiology of ETEC diarrhoeas.

INTRODUCTION

ENTEROTOXIGENIC Escherichia coli (ETEC) are one of the common aetiological agents of sporadic diarrhoea in both children and adults in different parts of the world. The pathogenic mechanism of diarrhoea caused by them involves two characteristics both of which are plasmid mediated, namely toxin production and fimbrial antigens. Theoretically any strain of E. coli can become enterotoxigenic by acquisition of the appropriate plasmids. However, in reality the majority of the strains of ETEC, either of human or of animal origin belong to a selected few "O" serogroups and to a certain extent K and H serotypes. In fact, certain investigators have even proposed identification of ETEC based on O, K and H antigens and biotypes only.

Another important feature of ETEC is the presence...
of fimbriae, K88, K99 and colonisation factor antigen (CFA), capable of mannose resistant haemagglutination of appropriate erythrocytes. These fimbriae have been considered essential for colonization of the small intestine by the ETEC.

In this report we present a strikingly different set of data on serogroups and fimbrial antigens of 309 strains of E. coli isolated from 274 cases of sporadic diarrhoea. The pattern is very different from whatever that has been reported so far. As a result, it is felt that the epidemiology of diarrhoea due to ETEC is quite different in these parts where bacterial diarrhoeas are very common.

**Experimental**

**Bacterial strains:** A total of 309 E. coli strains were isolated from 274 cases of sporadic diarrhoea in both children and adults admitted to Kasturba Hospital of Infectious Diseases, Bombay. In some cases more than one serogroup was encountered. Standard procedures for isolation and identification of Enterobacteriaceae were followed.

Five representative colonies of E. coli isolated from each case were studied.

**Serotyping:** All the E. coli isolates were sero-grouped at the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, India.

**Preparation of culture supernate for enterotoxicity assay:** A loopful of overnight tryptic-soy agar culture was inoculated into 10ml of Casamino acid yeast extract medium prepared as described by Evans, Evans and Gorbach and incubated on a rotary shaker (250 rpm) at 37°C for 18 hr. The bacterial cells were then separated by cold centrifugation at 1400 g for one hour. The resultant supernate was then used for toxin assay.

**Enterotoxicity assay:** Standard adult rabbit ileal loop technique was employed for detection of heat labile (LT) in the culture supernatants. For detection of heat stable toxin (ST) infant mouse technique of Dean et al. was employed. Each culture supernatant was tested in six mice. Since the purpose of this study was to identify toxigenic strains, only those strains which were LT negative were tested for ST. Thus, many LT producers could also have been positive for ST.

**Detection of fimbrial antigens:** Rapid slide agglutination technique of Evans et al. was employed. CFA/I was detected by the ability of the live bacterial suspension to agglutinate human group A red blood cells in the presence of mannose. CFA/II was detected by the ability of the live bacterial suspension to agglutinate bovine red blood cells in the presence of mannose.

**Statistical analysis:** Frequencies of isolation of strains belonging to different serogroups in relation to enterotoxicity and pilus antigen status were subjected to statistical analysis employing Poisson probability distribution.

**Results and Discussion**

**Incidence of enterotoxigenic strains:** Out of 309 E. coli isolates, as many as 189 (61.16%) were enterotoxigenic i.e. they either produced LT or ST or both, and 120 (38.84%) isolates were non-toxigenic.

**Relationship of pilus antigen and enterotoxicity:** The data on pilus status of E. coli isolates with respect to toxigenicity is presented in table 1. As many as 33.33% of enterotoxigenic strains were CFA positive i.e. they possessed either CFA/I or CFA/II. As expected, only 4% of the non-toxigenic strains were CFA positive. We suspect that even these might be toxigenic and would have been so identified, if a more sensitive test such as ELISA or Y1 adrenal cell assay was employed. Notable feature was that as many as 67% of the ETEC isolates were CFA negative. Of these 8.27% showed common pilus antigen. Thus, 58.73% of ETEC did not exhibit any haemagglutinating pili.

**Table 1**

Fimbriae and toxigenic characters of 309 E. coli isolates

<table>
<thead>
<tr>
<th>Fimbriae</th>
<th>Toxigenic (189)</th>
<th>Non-Toxigenic (120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA I or II</td>
<td>63 (33.33%)</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Type I pilus only</td>
<td>15 (7.94%)</td>
<td>3 (2.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>78/189 (41.27%)</td>
<td>8/120 (6.3%)</td>
</tr>
</tbody>
</table>

**Sero-group, pilus antigen and enterotoxicity:** Frequencies of predominant serogroups in non-toxigenic, toxigenic CFA positive, and toxigenic CFA negative isolates are shown in table 2. Altogether 47 different serogroups were encountered. Non-toxigenic strains belonged to just 28 of these and toxigenic belonged to 38 of these serogroups. The predominant serogroups isolated amongst non-toxigenic isolates were O1, O7, O12, O17, O20 and O60 at an average frequency of 6.5 isolates per serogroup. 27 were non-typhoid and the remaining 53 isolates were distributed amongst 22 different serogroups at an average frequency of 2.45.
TABLE 2

Frequency and characters of predominant E. coli isolates

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>01</th>
<th>02</th>
<th>07</th>
<th>011</th>
<th>017</th>
<th>020</th>
<th>060</th>
<th>NT*</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Toxigenic</td>
<td>8</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>8</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>Toxigenic CFA Positive</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Toxigenic CFA Negative</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>83</td>
</tr>
</tbody>
</table>

Frequencies greater than 3 are statistically significant—Poisson probability distribution.

NT*—Non-Typable.

The predominant serogroups encountered in ETEC isolates were 01, 02, 07, 011, 017 and 060 at an average frequency of 11.30 isolates per serogroup. Five strains were non-typable and the remaining 116 ETEC isolates belonged to 32 different serogroups at an average frequency of 3.6 per serogroup.

There was no evidence of temporal clustering of the serogroups.

The peculiar features in our study were.
1) More than 50% (67%) of ETEC strains were isolated without CFA in contrast to the reported incidences worldwide which is about 80%11 for CFA positive ETEC.
2) The predominant serogroups isolated amongst toxigenic E. coli were 01, 02, 07, 011, 017 and 060; none of which has so far been reported as serogroups characteristics of ETEC and are 06, 015, 025, 063, 078 and 01483.3.
3) The predominant serogroups isolated amongst non-toxigenic strains were the same as that isolated amongst toxigenic strains. One can only conclude one possibility that these non-toxigenic strains which were prevalent in community have become toxigenic by acquiring 'ent' plasmids and thus become the causative agent of diarrhoea in this population.
4) The incidence of ETEC diarrhoea was high (i.e. 56%) as compared to that reported in the west which is not more than 5%12,13. This finding is self-explanatory. Our country being under-developed, improper sanitary and hygienic conditions are predisposing factors for such high incidences of diarrhoea due to ETEC.

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