BACTERIAL PATHOGENICITY

Use of gene probes and adhesion tests to characterise Escherichia coli belonging to enteropathogenic serogroups isolated in the United Kingdom


Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT

Nine hundred and twenty-five Escherichia coli isolates from cases of diarrhoea in the United Kingdom and belonging to enteropathogenic E. coli (EPEC) O serogroups were examined for virulence properties. The tests included adhesion to HEp-2 cells, the fluorescence actin staining (FAS) test (which correlates with the ability to cause attaching and effacing lesions) and DNA hybridisations with probes to detect sequences for eaeA (E. coli attaching and effacing factor), EAF (EPEC adherence factor), verocytotoxins VT1 and VT2, enteroaggregative E. coli and diffusely adherent E. coli. The O serogroups examined were 18, 26, 44, 55, 86, 111, 114, 119, 125, 126, 127, 128 and 142. Six hundred and sixty strains (71.4%) hybridised with at least one of the DNA probes. Over 80% of strains in O serogroups 26, 55, 119, 125, 127 and 142 and 41% of strains of serogroups 86, 111, 114, 126 and 128 hybridised with the eae probe and most showed localised attachment and were FAS-positive. However, <10% of these eae probe-positive strains hybridised with the EAF probe. Eighty-four of 232 strains in O serogroups 44, 86, 111, and 126 were enteroaggregative. VT genes were detected in 57 of 402 strains in O serogroups 26, 55, 111 and 128. Identification of EPEC by serogrouping was shown to be an effective method of identifying strains with pathogenic potential, although the organisms were diverse in their properties.

Introduction

Enteropathogenic strains of Escherichia coli (EPEC) were first recognised to cause infantile enteritis in the 1940s. They were shown to belong to a restricted range of O serogroups and commercial antisera became available for their identification [1, 2]. Later it was shown that some strains of E. coli caused diarrhoea because they produced enterotoxins, or produced a verocytotoxin (VT), or were able to invade epithelial cells [3]. These groups of pathogenic E. coli were termed enterotoxigenic E. coli (ETEC), verocytotoxin producing E. coli (VTEC) or enteroinvasive E. coli (EIEC), respectively. However, characteristic EPEC strains isolated from outbreaks of diarrhoea did not belong to any of these groups, although occasionally strains belonging to EPEC serogroups and isolated from sporadic cases of diarrhoea did produce enterotoxins or VT [4].

A property possessed by typical EPEC strains was the ability to adhere in characteristic localised clusters to HEp-2 cells grown in tissue culture [5]. This adherence was shown subsequently to be accompanied by structural changes in the cells which resulted in the accumulation of polymerised actin beneath the attached bacteria, visualised by the use of fluorescein isothiocyanate-labelled phalloidin in the fluorescence actin staining (FAS) test [6]. These in-vitro tests with HEp-2 cells were shown to correlate with the ability of typical EPEC to cause attaching and effacing (AE) lesions of intestinal epithelial biopsy material. The ability of such EPEC strains to adhere well in vitro was shown to depend on the possession of a plasmid. An E. coli adherence factor (EAF) probe was developed from gene sequences carried on a plasmid [7]. Subsequently, chromosomal gene sequences were shown to be necessary for the formation of AE lesions as judged by the FAS test and the eae (E. coli attaching and effacing) probe was developed from the same E. coli strain [8]. In addition to the localised attachment (LA) shown by typical EPEC, other patterns of adhesion to HEp-2 cells have been recognised. These are the aggregative and diffuse patterns; strains
exhibiting these types of attachment have been termed
enteroaggregative _E. coli_ (EAggEC) and diffusely
adherent _E. coli_ (DAEC) [4].

Strains in EPEC O serogroups 18, 26, 44, 55, 86, 111,
114, 119, 125, 126, 127, 128 and 142 that were
isolated from cases of diarrhoea and received by the
Laboratory of Enteric Pathogens in the years 1985–
1993 were examined. They were tested for adhesion
to HEp-2 cells and for hybridisation with several probes
used to identify the different classes of diarrhoeagenic
_E. coli._

**Materials and methods**

**Bacterial strains**

The strains were received from laboratories in many
different areas of the United Kingdom. They were
isolated from the faeces of patients of all ages with
diarrhoea during the period 1985–1993; 867 (94%) of
the 925 strains were from children <4 years old. The
strains were identified and serotyped with antisera to O
antigens 1–173 and H antigens 1–55 [9]. Some of the
strains have been included in previous studies [10–12].

**DNA hybridisation**

Strains were tested by colony hybridisation [13]. The
VT1 probe was a 0.75-kb _HincII_ fragment derived
from strain H19 ( _E. coli_ 026:H11) and the VT2 probe
was a 0.85-kb _AvaI–PstI_ fragment derived from strain
E32511 ( _E. coli_ 0157:H–) [14]. The VT probes were
labelled with fluorescein-dUTP or digoxigenin and
used as described previously [15, 16]. The _eae_ and
EAF probes were derived from strain E2348/69 [7, 8].
The EAggEC probe was that described by Baudry _et al._
[17] and the DAEC probe was a 370-bp _PstI_ fragment
within the _daaC_ gene of strain C1845 [18]. The _eae_,
EAF, EAggEC and DAEC probes were labelled with
fluorescein-dUTP. Hybridisation and washing were
under stringent conditions with the following excep-
tions. For the EAggEC probe, membranes were washed
twice at room temperature for 15 min in 2 × SSC, SDS
0.1% and then in 5 × SSC, SDS 0.1% for 15 min at
54°C followed by 30 s at room temperature in
2 × SSC, SDS 0.1%. Membranes from DAEC probe
tests were washed twice at room temperature for
15 min in 2 × SSC, SDS 0.1% and then in 0.5 × SSC,
SDS 0.1% at 68°C for 15 min.

**Adhesion tests**

Adhesion to HEp-2 cells was tested in the presence of
D-mannose 1% w/v as described previously [19]. The
pattern of adhesion was assessed as localised, diffuse or
aggregative at the end of a 6-h test. All the strains that
showed no adhesion were tested in at least two
experiments. The FAS test was that of Knutton _et al._
[6].

**Results**

**Testing of strains with DNA probes**

Of the 925 _E. coli_ strains isolated in the UK and
belonging to 13 O serogroups tested for hybridisation
with the _eae_, EAF, EAggEC, DAEC and VT probes,
660 (71.4%) strains hybridised with at least one probe
(Table 1).

The largest number of strains (520) hybridised with
the _eae_ probe. Within each serogroup the positive
strains were usually of particular O:H combinations
and there were also some non-motile strains. The _eae-
positive_ serotypes most commonly found were
O111:H25, O119:H2, O125:H6, O127:H40, O127:H45,
O127:H–, O128:H2 and O128:H8. These 13 serotypes accounted for 80% of the _eae-positive_ strains although there was a total of 51 _eae-positive_ serotypes identified. The _eae_ genes were found in all
serogroups except O18 and O44. Results of the EAF
probe tests showed that 49 strains were positive and
all except one O18:H4 strain also hybridised with the
_eae_ probe.

One hundred and twenty-one strains were EAggEC-
probe positive. All these strains gave negative results
with the _eae_ probe and the majority belonged to
distinct O:H serotypes such as O44:H18, O111:H21,
O126:H27 and O128:H35. Some of the EAggEC
probe-positive strains also hybridised with the DAEC
probe as observed in a recent study of O44:H18
strains [20]. In addition to these strains that hybridised
with both the EAggEC and DAEC probes, there were
10 strains that hybridised with the DAEC probe and
not the EAggEC probe (Table 1). Four of these 10
DAEC probe-positive strains, all of which were of
serotype O55:H–, were unusual in that they hybrid-
ised with the _eae_ probe.

VT genes were detected in 57 strains of serogroups
O26, O55, O111 and O128; 41 of these VTEC strains
were in serogroup O26 and most were O26:H11, as
found in previous studies [12, 21]. Forty-five of the 57
VTEC strains also hybridised with the _eae_ probe and
the remaining 12 _eae-negative_ strains were in
serogroup O128.

Two hundred and sixty-five strains did not hybridise
with any of the probes and 90 of these belonged to
serogroup O18 (Table 2). The remaining probe-nega-
tive strains belonged to all 12 serogroups but there
were no more than eleven strains of any individual
O:H serotype.

**Adhesion to cell cultures**

Selected strains were examined for attachment to HEp-
2 cells and the results were correlated with those of the
hybridisation tests (Tables 3 and 4). All 47 strains
Table 1. Properties of strains that hybridise with DNA probes

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Number positive/total tested</th>
<th>Number of strains</th>
<th>Hybridisation with</th>
<th>eae</th>
<th>EAF</th>
<th>EAggEC</th>
<th>DAEC</th>
<th>VT</th>
<th>H type (number)</th>
</tr>
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<td>1</td>
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<td>123/128</td>
<td>81</td>
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<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
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<td>8(2), 11(18), (−61)</td>
</tr>
<tr>
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<td>41</td>
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<td>+</td>
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<td></td>
<td>+</td>
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<td>11(35), 21(1), 32(1), (−4)</td>
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<td>2(1)</td>
</tr>
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<td>+</td>
<td></td>
<td>(−4)</td>
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</tr>
<tr>
<td>O119</td>
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<td>5</td>
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</tr>
<tr>
<td>O125</td>
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<td>9(2)</td>
</tr>
<tr>
<td>O126</td>
<td>49/79</td>
<td>4</td>
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<td>9</td>
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<td>+</td>
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<td>2(7), 33(1), 43(1)</td>
</tr>
<tr>
<td>O127</td>
<td>55/69</td>
<td>50</td>
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<td>4(1), 40(13), 45(12), (−24)</td>
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<td>O128</td>
<td>104/140</td>
<td>76</td>
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<td>+</td>
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<td>2(54), 8(12), 11(1), 12(1), 47(1), (−7)</td>
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<td>+</td>
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<td>2(11), (−1)</td>
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<td></td>
<td>12</td>
<td></td>
<td>+</td>
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<td>2(11), (−1)</td>
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<td>7(1), 35(11)</td>
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<tr>
<td>O142</td>
<td>20/24</td>
<td>16</td>
<td></td>
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<td></td>
<td></td>
<td>6(2), 10(1), 23(1), 34(6), 35(2), 36(2), 52(1), (−1)</td>
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<td>+</td>
<td></td>
<td></td>
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<td>4(1), 6(3)</td>
</tr>
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</table>

Examined that were eae- and EAF-probe-positive showed localised adhesion, although there was considerable variation in the percentage of HEp-2 cells with adherent bacteria. Of the 321 strains that hybridised with the eae probe but were EAF-negative, 201 showed some LA. The percentage of cells that showed LA ranged from 1 to 92 and was usually lower than adhesion observed with the eae-positive, EAF-

Table 2. Flagellar (H) types of strains that did not hybridise with probes

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Total</th>
<th>H types (number)</th>
<th>Number of non-motile strains</th>
</tr>
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<tr>
<td>O18</td>
<td>90</td>
<td>1(6), 4(2), 5(3), 7(41), 8(1), 14(2), 49(1), 34</td>
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</tr>
<tr>
<td>O26</td>
<td>5</td>
<td>32(4)</td>
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<tr>
<td>O44</td>
<td>7</td>
<td>5(1), 18(5), 41(1)</td>
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</tr>
<tr>
<td>O55</td>
<td>20</td>
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<td>0</td>
</tr>
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<td>O86</td>
<td>15</td>
<td>4(1), 8(1), 10(1), 18(1), 20(1), 27(2), 30(2), 43(1)</td>
<td>5</td>
</tr>
<tr>
<td>O111</td>
<td>13</td>
<td>12(3), 19(1), 45(4)</td>
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</tr>
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<td>O114</td>
<td>16</td>
<td>4(6), 9(4), 10(1), 25(2)</td>
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</tr>
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<td>2</td>
<td>4(1)</td>
<td>1</td>
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<td>O125</td>
<td>13</td>
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<td>O126</td>
<td>30</td>
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<td>4(2), 5(1), 21(2), 40(6)</td>
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<td>O128</td>
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<td>1(9), 2(1), 9(1), 12(5), 19(1), 27(1), 35(6), 45(1), 46(1), 47(1), 49(1)</td>
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<td>O142</td>
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<td>4(1), 11(1), 38(2)</td>
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Table 3. Examination of eae-positive strains and correlation with EAF probe and cell tests

<table>
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<th>Serogroup</th>
<th>H type (n)</th>
<th>Total</th>
<th>EAF probe</th>
<th>Percentage of cells with LA*</th>
<th>Number of strains with no attachment</th>
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<tr>
<td></td>
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<td>Mean</td>
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<td>O26</td>
<td>–</td>
<td>24</td>
<td>–</td>
<td>1–32</td>
<td>9.5</td>
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<td>11</td>
<td>18(13VT+)</td>
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<td>1–52</td>
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<td>32</td>
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<td>–</td>
<td>1–52</td>
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<td>52(1) –(1)</td>
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*A A selection of LA strains of all serotypes was FAS positive. The eae DA O55 strains were FAS negative.
†These four strains hybridised with the eae and DAEC probes.

Discussion

Over 70% of the strains from the 13 EPEC serogroups hybridised with at least one of the DNA probes for virulence factors or putative virulence factors. The eae property was found in >80% of strains belonging to O serogroups 26, 55, 119, 125, 127 and 142 and in >40% of strains of O serogroups 86, 111, 114, 126 and 128. The majority of these eae probe-positive strains gave LA and were FAS test positive. However, >90% of these eae probe-positive strains were unlike typical EPEC in that they did not hybridise with the EAF probe. Some of the EAF-positive strains belonged to serotypes usually associated with outbreaks of infantile gastroenteritis such as O55:H–, O114:H2, O119:H6, O126:H2, O127:H6, O128:H2 and O142:H6. The majority of the EAF-negative strains belonged to serotypes that were different from those of the EAF-positive strains. Therefore, it is unlikely that the
Table 4. Correlation of EAggEC and DAEC probes with cell tests

<table>
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<tr>
<th>Serogroup</th>
<th>H type (n)</th>
<th>Number of strains</th>
<th>EAggEC</th>
<th>DAEC</th>
<th>Adhesion type</th>
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<td>+</td>
<td>+</td>
<td>Agg</td>
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<td>+</td>
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<td>1</td>
<td>+</td>
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</table>

EAF-negative strains resulted from loss of the EAF plasmid from positive strains.

Although most eae probe-positive strains showed LA, the lack of attachment in some cases may suggest that there is no or very poor expression of the eaeA gene or other genes. Recent studies have shown that the EAF plasmid controls the expression of the eaeA gene product, a 94-kDa protein termed intimin [22]. The perA locus, a plasmid encoded regulator, increases expression of intimin by 100-fold and this perA gene has been detected in all EPEC strains that carry the EAF plasmid. In this study, all the eae-positive, EAF-positive strains expressed LA and were FAS test-positive. The presence of the perA locus or a similar regulatory gene in the eae-positive, EAF-negative strains is not yet known. The EAF plasmid also carries the bfpA gene that encodes bundle-forming pili (BFP) and it has been suggested that these pili are an adhesin of EPEC [23]. The bfpA and EAF probes were correlated in a recent study [24] and this demonstrated that the bfpA probe hybridised with 137 of 138 EAF-positive strains and only with 10 EAF-negative strains. Hybridisation of the eae-positive, EAF-negative strains examined in the present study with a bfpA probe would provide further information on the distribution of these pili in EPEC.

Many strains belonging to O serogroups 44, 86, 111 and 126 were enteroaggregative by probe and adherence tests and in a total of nine serogroups there were EAggEC probe-positive strains. This extends the range of EPEC O serogroups that contain EAggEC [4, 11, 20, 25] and supports the suggestion that EAggEC may be a significant cause of diarrhoea in Britain. Previous studies demonstrated that the aggregative pattern of adherence is usually plasmid determined [25] and in strain 17-2, serotype O3:H2, plasmid-encoded genes specified bundle-forming fimbriae on the surface [26]. At present it is not clear whether EAggEC strains of different serotypes specify antigenically distinct adhesins. Tests suitable for clinical diagnostic laboratories need to be developed so that the importance of EAggEC can be assessed.

There has been some controversy over the virulence of EPEC and many laboratories have discontinued testing for EPEC [4, 27]. The results of this study have demonstrated that identification of EPEC by serogrouping is an effective method of identifying strains of E. coli with pathogenic potential, although the organisms were diverse in their properties. In particular, the virulence of eae-positive, EAF-negative strains and of EAggEC strains needs further evaluation.

References

6. Knutton S, Baldwin T, Williams PH, McNeish AS. Actin


