

TRADITIONAL AND NON-TRADITIONAL ENTEROPATHOGENIC *ESCHERICHIA COLI* SEROGROUPS

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CATEGORIES OF DIARRHEAGENIC *E. COLI*

There are at least seven categories of diarrheagenic *E. coli* that are now recognized: enteropathogenic *E. coli* class I (EPEC class I), enteropathogenic *E. coli* class II (EPEC class II), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffusely adhering *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). The main characteristics that define each one are shown in Table 1. The division of EPEC into class I and class II distinguishes strains with the EAF (EPEC adherence factor) plasmid from those without the plasmid. They are provisional terms while we do not have a definition for EPEC. The seventh diarrheagenic *E. coli* category (EIEC), although shown in Table 1, never occurs in the EPEC serogroups.

TRADITIONAL EPEC SEROGROUPS

As there is no consensus, we will consider as traditional EPEC serogroups the ones listed by WHO in 1987 (31): O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158.

OCCURRENCE OF DIFFERENT CATEGORIES OF DIARRHEAGENIC *E. COLI* IN THE EPEC SEROGROUPS

The main purpose of this presentation is to show that the EPEC serogroups almost always include bacteria of different categories of diarrheagenic *E. coli* with distinct mechanisms of pathogenesis. In the first part of this presentation, we will review the relevant EPEC literature and in the second part we will show recently obtained data from our laboratories on the clonal diversity of strains of EPEC serogroups O111,

O119 and O55, which are the most frequent serogroups recovered from diarrheal disease in Brazil (27, 28, 29). At the end, we will discuss briefly the characteristics of strains of non-traditional EPEC serogroups.

Serogroup O26

In this serogroup, class I EPEC are rarely recovered. Interestingly, the first strain implicated in infantile diarrhea (F41) which belonged to serotype O26:H⁻ and was identified by Ørskov in 1951 (11) has recently been classified as class II EPEC by Robins-Browne (16). Many studies on O26 strains have shown that non-motile strains typically have the intimin-encoding *eaeA* gene (*eaeA*+), whereas strains with the H11 flagellar antigen are typically *eaeA*+ and produce verocytotoxin VT1 (22, 30). Usually, these O26:H11 strains harbor the EHEC plasmid (22). Apparently, there are geographic differences in the virulence of the O26:H11 strains because in Brazil, strains of this serotype do not produce verotoxins (17, 24). Serotype O26:H32, which is isolated with relative high frequency, lacks these virulence factors (17).

Serogroup O55

Several authors have observed that, among O55 strains, the presence of the EAF plasmid is correlated with serotype O55:H6 whereas O55:H7 strains generally do not carry the EAF plasmid (8,10,18,23,30). Therefore, O55:H6 is EPEC class I and O55:H7 is EPEC class II. Recently, Scotland *et al.* (23) have shown that strains of serotypes O55:H10 and O55:H27 may have characteristics of EAggEC.

Table 1 - Main characteristics of diarrheagenic *E. coli*

Categories ^a	Main characteristics ^b
EPEC Class I	<i>eaeA</i> gene, plasmid EAF
EPEC Class II	<i>eaeA</i> gene
EHEC	<i>eaeA</i> gene, production of SLT-I and/or SLT-II
ETEC	production of LT and/or ST
EAggEC	aggregative adherence pattern
DAEC	diffuse adherence pattern
EIEC	invasion of tissue culture cells, production of keratoconjunctivitis in guinea-pig

^a See text for definitions.

^b *eaeA*, EPEC attachment/effacement gene; SLT-I and SLT-II, Shiga-like toxins; LT, heat-labile enterotoxin; ST, heat-stable enterotoxin.

Serogroup O86

Serotype O86:H34 strains have been identified as EPEC class I by several authors (5,12) and Robins-Browne *et al.* (16) identified the archetypal strains E990 (O86:H⁻) and F1961 (O86:H2) as EPEC class I and EAggEC, respectively.

Serogroup O111

This serogroup is the most extensively studied EPEC serogroup and it has been shown that O111 strains belong to divergent clones with distinct combinations of virulence properties, including EPEC class I strains (serotypes O111:H2 and O111:H⁻), EPEC class II (serotype O111:H25), EHEC (serotypes O111:H8 and O111:H⁻) and EAggEC (serotype O111:H21) (5,8,9,13,20).

Serogroup O114

Beutin *et al.* (2) examined 88 strains of this serogroup for virulence markers, O:H serotypes, and electrophoretic types (ET) by multilocus enzyme electrophoresis. ETs 14 and 15 (serotype O114:H2) were found to be EPEC class I, ET 5 (serotype O114:H4), EHEC, and ETs 2 and 3, ETEC. The majority of the strains lacked virulence determinants for enteric infections.

Serogroup O119

The few data on this serogroup show that the serotypes O119:H6 and O119:H⁻ are EPEC class I (5,10).

Serogroup O125

Ørskov & Ørskov (12) mention that serotype O125:H6 is EPEC class I and Scotland *et al.* (21) have shown that strains of this serotype react with the EAF probe.

Serogroup O126

Yam *et al.* (32) examined 39 strains of this serogroup by different methods and found out that 31 were ETEC (serotype O126:H12) and one strain (serotype O126:H10) was EAggEC. The remaining seven strains harbored no known virulence factors for diarrheagenic *E. coli*. Scotland *et al.* (20) identified O126:H7 strains as EAggEC.

Serogroup O127

The existing information on this serogroup indicates that serotypes O127:H⁻ and O127:H6 are EPEC class I (5,10).

Serogroup O128

Studies carried out in England by Scotland *et al.* (19) and Smith *et al.* (25) and also in Brazil by Guth *et al.* (6) demonstrate that this serogroup is extremely complex, since it includes all categories of diarrheagenic *E. coli*. Serotyping alone seems not to differentiate the diarrheagenic *E. coli* categories in this serogroup.

Serogroup O142

So far, only EPEC class I has been reported in this serogroup (5, 12).

Serogroup O158

No studies on the virulence factors were found in the literature. Three strains tested in our laboratories did not harbor any of the known virulence factors of diarrheagenic *E. coli*.

CLONAL ANALYSIS OF SEROGROUPS O111, O119 AND O55

We have examined, in the last two years, a large number of strains of serogroups O111, O119 and O55, isolated in Brazil and in other parts of the world, and characterized them by electrophoretic types by

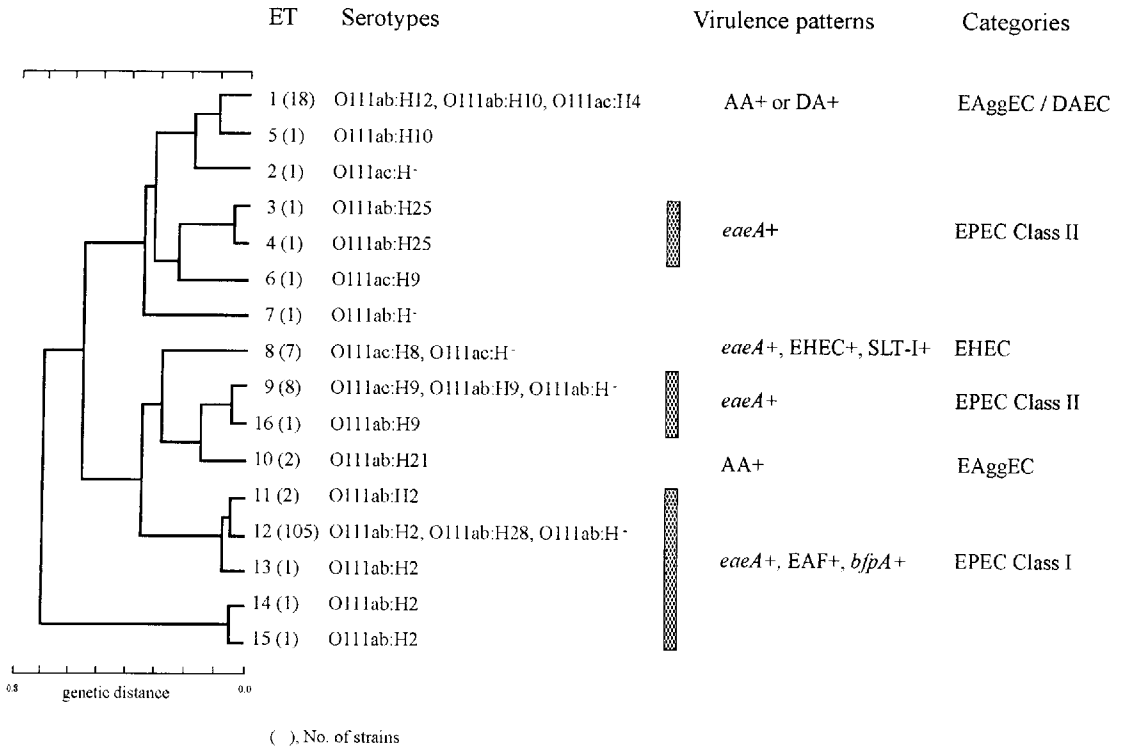


Figure 1 - Dendrogram of genetic relationship of 16 ETs of *E. coli* O111 strains.

multilocus enzyme electrophoresis, O and H antigens, and virulence factors for diarrheagenic *E. coli*, both by DNA probes, adherence tests on cultured cells (HEp-2) and the FAS test. The methods used were described elsewhere (4).

Serogroup O111

We have studied 152 strains of this serogroup, which belonged to several O:H types and were isolated in the last 30 years, in different parts of the world. The results obtained are shown in Fig. 1. Sixteen ETs marking bacterial clones were identified among the 152 strains. Clones ET1, ET8, ET9 and ET12 were predominant.

Clone ET1. This clone includes serotypes O111ab:H12 (14 strains), O111ab:H10 (3 strains) and O111ac:H4 (1 strain). Most ET 1 strains exhibited the aggregative adherence pattern but only the O111ab:H10 strains reacted with the probe for this pattern. While very little is known about the role of serotypes O111ac:H4 and O111:H10 in diarrhea, the role of serotype O111ab:H12 is well established. In fact, this serotype was one of the first *E. coli*

epidemiologically implicated in diarrhea (7) and has been found in association with diarrhea by many authors (3). This is the first report on the virulence properties of this serotype.

Clone ET8. All strains of this clone had O antigen of subgroup "ac" and, with exception of one strain that had the H8 antigen, the others were non-motile. All strains were *eaeA* positive and six of the seven strains studied reacted with the probe for SLT-I and produced SLT-I toxin in titers of 10⁴ in Vero cells. The supernatants of the seven strains produced fluid accumulation in ligated ileal loops, a fact that has not been demonstrated so far. In view of these characteristics, clone ET8 may be considered as EHEC.

Clone ET9. The strains of this clone were non-motile (H⁻) or presented the H9 antigen. All strains reacted with the *eaeA* probe but none produced SLT toxins. Also they did not produce fluid accumulation in ligated ileal loops. Most strains produced enterohemolysin. As none of the strains has the EAF plasmid, they were considered as EPEC class II. The role of this kind of strain in diarrhea is unknown.

Clone ET10. The two strains of this clone reacted with the probe for AA (aggregative adherence) and exhibited the aggregative adherence pattern. Therefore, they are different from most of aggregative ET1 strains which did not react with the AA probe. *E. coli* O111:H21 has also been found to be aggregative by Scotland et al. (20).

Clone ET12. All strains in this clone had the EAF plasmid and so were considered EPEC class I. Approximately 40% of these strains were non-motile and 60% had the H2 antigen. On the basis of these results, we conclude that O111 strains comprise at least 4 diarrheagenic *E. coli* categories, namely, EAggEC, EHEC, EPEC class I and EPEC class II. It should be noted that strains without virulence factors did not occur in serogroup O111 and that most (65%) of O111 strains are EPEC class I.

Serogroup O119

We have studied 84 strains of this serogroup and identified 11 clonal types (Fig. 2). Clone 1 was represented by 52 strains which had the H6 antigen

or were non-motile. Serotype O119:H6 was also found in most of the other clones and with a few exceptions, strains of this serotype reacted with probes for *eaeA*, *bfpA* and EAF. So, this serotype corresponds to the typical or class I EPEC. Interesting enough, serotype O119:H2 strains, although reacting with the *eaeA* and *bfpA* probes, did not react with the EAF probe. Furthermore, although they reacted with the *bfpA* probe, these strains do not express the BFP fimbriae.

Serogroup O55

We have studied 71 O55 strains and identified 8 ETs or clones (Fig. 3). However, most of the strains belonged to clone 1 (29 strains) and 5 (23 strains). Clone 1 strains had H6 antigens or were non-motile. Most of them reacted with the probes for *eaeA*, EAF and *bfpA* and so corresponded to EPEC class I. Clone 5 strains had H7 antigen or were non-motile and showed 5 patterns of virulence genes, including strains having at the same time the genes for diffuse adherence, BFP and EAF. These strains exhibited the

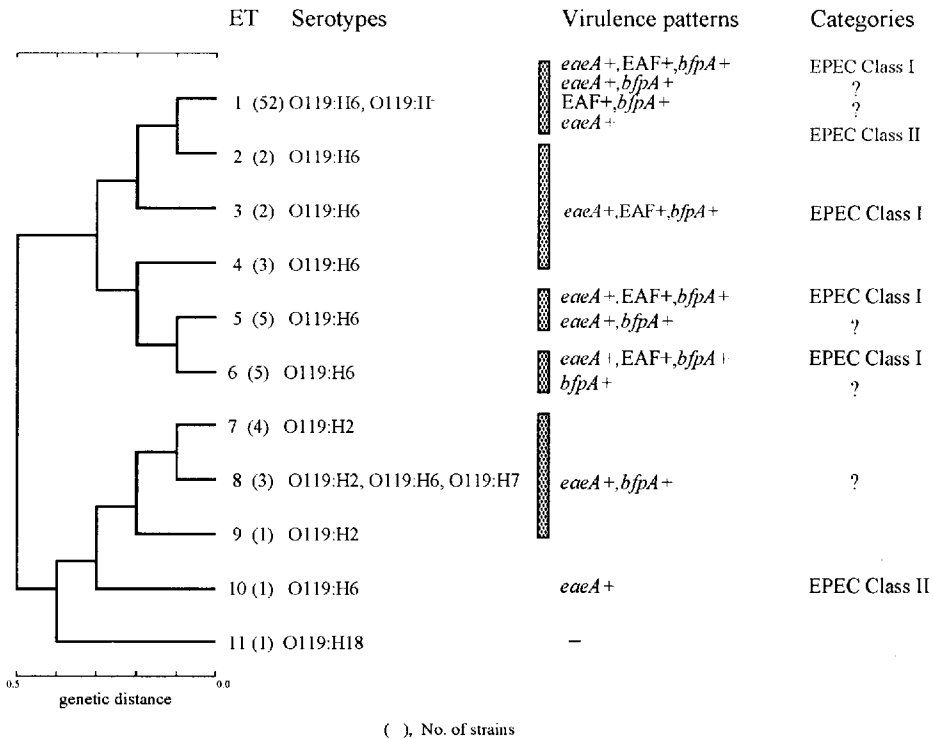


Figure 2 - Dendrogram of genetic relationship of 11 ETs of *E. coli* O119 strains.

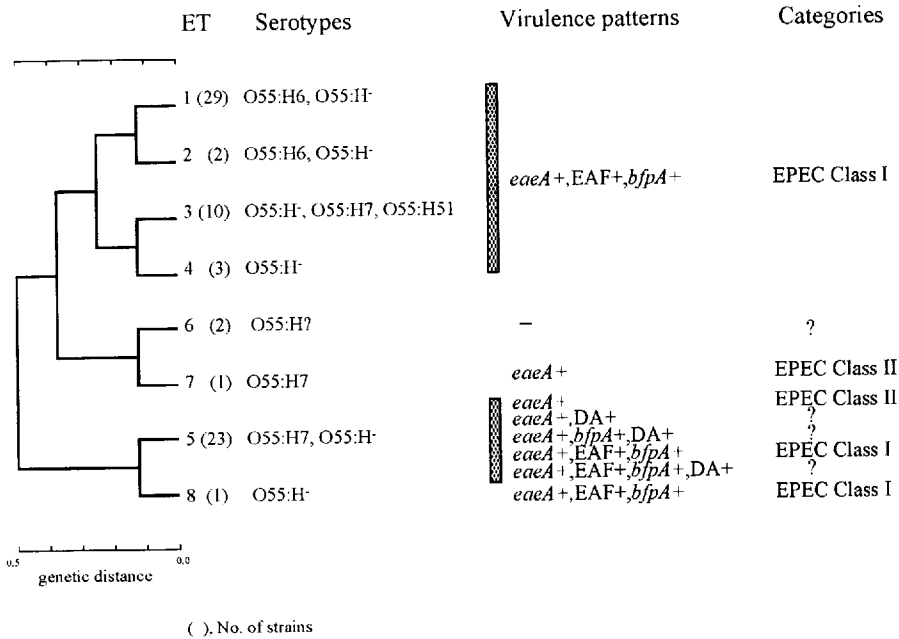


Figure 3 - Dendrogram of genetic relationship of 8 ETs of *E. coli* O55 strains.

localized and the diffuse adherence pattern simultaneously. To interpret these patterns, we hypothesize that the most common characteristics of a clone or a clone complex provide clues of the ancestral condition and wildtype configuration of the pathogen. For example, the *eaeA* variants in clones 1 and 3 are presumably derived from the most common virulence genotype (*eaeA*-EAF-*bfpA*) by the loss of the EAF plasmid in nature, during the course of an infection, or in culture. In contrast, *eaeA* alone is the most common virulence gene in clone 5. In this case, we posit that wildtype clone 5 strains have acquired the EAF or the DA plasmid or both in the natural population. The acquisition of virulence plasmids could be more likely to occur in environments like Brazil where EAF+ EPEC strains are prevalent. Alternatively, it is possible that the ET 5 clone typically carries these plasmids, which are then lost in nature or in the laboratory.

NON-TRADITIONAL EPEC SEROGROUPS

Regarding the non-traditional EPEC serogroups, we should mention that typical EPEC strains have been found in such serogroups as O2, O15, O88 and

many others. Apparently they may occur in many *E. coli* serogroups (1,14). On the other hand, such serogroups as O18, O20 and O44, considered as EPEC, have proved to be non-virulent (O18 and O20) or to be EAggEC (O44) (15,26).

CONCLUSIONS

1. The EPEC serogroups are very heterogeneous;
2. Most of them contain EPEC class I serotypes in association with other categories of diarrheagenic *E. coli*;
3. In some serogroups the EPEC serotypes are prevalent, as it happens with O55, O111, O119 and others. In other serogroups, such as O125 and O126, other categories of diarrheagenic *E. coli* predominate.
4. The most frequent diarrheagenic *E. coli* categories after EPEC class I are EPEC class II and EAggEC.

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