浙江省腹泻患者致泻性大肠埃希菌血清型分布及其鉴定效率的评价

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【摘要】目的 了解浙江省致泻性大肠埃希菌(DEC)血清型分布并探讨其血清学鉴定分类方法的鉴定效率。方法 对2009年7月至2013年6月浙江省腹泻症候群病原谱监测网络菌株库中的696株DEC菌株(通过毒力基因鉴定)开展血清学凝集试验,比较毒力基因和血清学鉴定分类的结果。结果 696株DEC中288株(41.4%)能明确O抗原型别,分属于35种O血清群。171株(24.6%)H血清凝集,分属于21种H型。肠集聚性大肠埃希菌(EAEC),产肠毒素性大肠埃希菌(ETEC),肠致病性大肠埃希菌(EPEC)和肠出血性大肠埃希菌(EHEC)凝集率分别为31.9%(130/408),70.6%(127/180),31.5%(29/92)和14.3%(2/14),分属于30,18和15种O血清群,1株EHEC为O157:H7。EAEC和EPEC血清群相对较多样化,而ETEC则相对集中,不同类型DEC可具有同一血清群/型。根据血清学结果可分类的75株DEC中,42株毒力基因和血清学分类结果一致,33株不一致。结论 浙江省DEC血清群/型种类多样,单纯采用血清学筛查可造成极大的漏检或误分,建议采用毒力基因鉴定分类。

【关键词】致泻性大肠埃希菌;毒力基因;血清型

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【Abstract】Objective To investigate the serotypes of Diarrheagenic Escherichia coli (DEC) isolated from diarrheal patients in Zhejiang province and to explore the identification efficiency of serological screening methods. Methods Serological agglutination tests were carried out in 696 strains of DEC (through the identification of virulence genes) which were selected from the Infectious Diarrheal Pathogen Monitoring Network Strain Bank of Zhejiang province, from July 2009 to June 2013. Results of virulence genes, serological identification and classification were compared. Results Among the 696 isolates of DEC, O antigen type was identified in 288 (41.4%) isolates which belonging to 35 different ‘O’ serum types. H antigen was seen in 171 (24.6%) isolates and determined as having 21 types. The agglutination rates of EAEC, ETEC, EPEC and EHEC isolates were 31.9% (130/408), 70.6% (127/180), 31.5% (29/92) and 14.3% (2/14), respectively and belonged to 30, 18, 15 kinds of ‘O’ sero-groups, respectively. One EHEC isolate was identified as O157:H7. Serum groups were diverse for EAEC and EPEC, while relatively concentrated on ETEC. Different types of DEC might belong to the same sero-group or type. Among the 74 strains of DEC available for classification serologically, 41
isolate were in consistent with virulence gene identification and another 33 strains were not. 

**Conclusions** The sero-group/type of DEC strains in Zhejiang were varied. Based on the serological screening method alone, DEC classification might end in getting the wrong answer, thus we would recommend the use of virulence gene for the purpose of identification.

**Key Words** Diarrheagenic Escherichia coli; Virulence gene; O/H serotypes 

**Fund Program:** National Science and Technology Major Project (2012ZX10004-210)

**Materials and Methods**

1. **Bacterial** 
From 2011 to 2013 in June, 2013, the 696 stool specimens (1127 specimens) from the enteric E. coli (EIEC) strains from 931 stool specimens, 696 stool specimens from 931 stool specimens were isolated and identified. The 1127 stool specimens were isolated from 931 stool specimens and identified as different serotypes and 1127 stool specimens were isolated from 931 stool specimens and identified as different serotypes.

2. **Method**

(1) **Virulence Gene Detection.** The amplified fragments were analyzed by gel electrophoresis, and the amplified fragments were shown in Figure 1A (EHEC). The virulence gene profile of the 696 stool specimens was shown in Table 1.

(2) **Serotypes.** The serotypes of the 696 stool specimens were showed in Table 2. The stool specimens were divided into 1127 stool specimens and 696 stool specimens. The 1127 stool specimens were divided into 876 stool specimens and 727 stool specimens. The 696 stool specimens were divided into 876 stool specimens and 727 stool specimens. The 1127 stool specimens were divided into 876 stool specimens and 727 stool specimens. The 696 stool specimens were divided into 876 stool specimens and 727 stool specimens. The serotype of the stool specimens was showed in Table 2.

3. **Statistical Analysis.** The data were analyzed using SPSS 13.0 software. The categorical data were compared using the chi-square test. The continuous data were compared using the t-test. The differences were considered significant when *P* < 0.05.

**Results**

1. **Virulence Gene Detection.** The amplified fragments were analyzed by gel electrophoresis, and the amplified fragments were shown in Figure 1A (EHEC). The virulence gene profile of the 696 stool specimens was shown in Table 1.

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### 表1 浙江省696株DEC毒力基因和血清型分布

<table>
<thead>
<tr>
<th>菌株</th>
<th>菌株数 (构成,% )</th>
<th>毒力基因(株数)</th>
<th>血清型(株数)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHEC</td>
<td>14(2.0)</td>
<td>stx2 + excV(6), stx2(5), stx1 + excV(2), stx1(1)</td>
<td>O157: H7 (1), O1: H (1), O: H (12)</td>
</tr>
<tr>
<td>EIEC</td>
<td>1(0.1)</td>
<td>invE(1)</td>
<td>O: H (1)</td>
</tr>
<tr>
<td>EAEc/ETEC</td>
<td>1(0.1)</td>
<td>astA + agrR + el(1)</td>
<td>O: H (1)</td>
</tr>
</tbody>
</table>

注：EPEC为肠病原性大肠埃希菌，EHEC为肠出血性大肠埃希菌，ETEC为产肠毒素性大肠埃希菌，EIEC为肠侵袭性大肠埃希菌；通过毒力基因鉴定分型，该菌株同时携带EAEc和ETEC的毒力基因，记为EAEc/ETEC

血清凝集(其中62株O血清未凝集)，分属于21种H型，其余菌株O血清和H血清均未发生凝集。居首位的血清型为O6：H16(21株，其中4株为EAEc，17株为ETEC)和O159：H34(20株，全部为ETEC)。常见O血清群为O159(43株，6.2%)，O6(43株，6.2%)，O148(28株，4.0%)，O45(25株，3.6%)，O25(22株，3.2%)和O15(21株，3.0%)；H血清群常见的为H16(31.4%，4.5%)，H34(25.3%，3.6%)，H18(16株，2.3%)，H2(16株，2.3%)和H41(14株，2.0%)。

EAEc O血清凝集型为31.9%(130/408)，分属于30种O血清群，其中36.9%集中在O6(17)，O15(16)和O45(15)；ETEC凝集型为70.6%(127/180)；分属于18种O血清群，其中75.8%集中在O159(39)，O6(25)，O148(23)和O25(13)；EPEC凝集型为31.5%(29/92)，分属于15种O血清群，其中O45和O1分别有8株和5株；14株EHEC中仅2株凝集，1株为O157：H7，另1株为O1群(表1.2)。

3. 毒力基因鉴定和血清学分类结果的比较：根据《感染性腹泻诊断标准》(标准)中的血清学分类方法，本研究仅能对75株(10.7%，75/696)DEC分类，其中ETEC 40株，EAEc 28株，EPEC 6株和EHEC 1株。75株中有42株(56.0%)经毒力基因检测方法分类结果和血清学分类结果一致，33株(44.0%)不一致(表2)。其中可分类的40株ETEC中有36株血清学分类仍为ETEC (O159：H34，O148：H28，O169：H和O27：H7)。33株不一致的菌株中有29株毒力基因鉴定为EAEc，4株为ETEC，但血清学分类多为EPEC(图1)。

### 讨 论

目前临床实验室针对腹泻患者一般仅开展霍乱弧菌、沙门菌和志贺菌病原学筛查，少数实验室开展副溶血弧菌和大肠埃希菌O157：H7的鉴定，但未对DEC其他型别开展筛查。实际上，DEC引发的肠道感染在肠道病原谱中构成比例超过了志贺菌和沙门菌，成为婴幼儿和成人腹泻重要致病菌。本课题组发现浙江地区门诊腹泻患者DEC的检出率为18.8%，远远超过传统常规筛查的病原检出率，其中以EAEc、ETEC和EIEC为主，EHEC和EIEC较少见。通过多重PCR方法检测DEC的毒力基因进行分类，是目前病原监测实验室常用方法，具有灵敏、简便、成本较低等特点。
### 表2 浙江省696株DEC血清学凝集率和血清学鉴定分类

<table>
<thead>
<tr>
<th>分类</th>
<th>株数</th>
<th>O血清凝集株数(率,%)</th>
<th>H血清凝集株数(率,%)</th>
<th>血清学鉴定株数(率,%)</th>
<th>分类不一致株数(构成比,%)</th>
<th>分类一致株数(构成比,%)</th>
<th>合计</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAEC</td>
<td>408</td>
<td>130(31.9)</td>
<td>76(18.6)</td>
<td>28(6.9)</td>
<td>28(100.0)</td>
<td>0(0.0)</td>
<td>696</td>
</tr>
<tr>
<td>ETEC</td>
<td>180</td>
<td>127(70.6)</td>
<td>71(39.4)</td>
<td>40(22.2)</td>
<td>4(10.0)</td>
<td>36(90.0)</td>
<td>260</td>
</tr>
<tr>
<td>EPEC</td>
<td>92</td>
<td>29(31.5)</td>
<td>23(25.0)</td>
<td>6(6.5)</td>
<td>1(16.7)</td>
<td>5(83.3)</td>
<td>147</td>
</tr>
<tr>
<td>EHEC</td>
<td>14</td>
<td>2(14.3)</td>
<td>1(7.1)</td>
<td>1(7.1)</td>
<td>0(0.0)</td>
<td>1(100.0)</td>
<td>28</td>
</tr>
<tr>
<td>EIEC</td>
<td>1</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>EAEC/EPEC</td>
<td>1</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

注: EPEC为肠致病性大肠埃希菌; EHEC为肠出血性大肠埃希菌; ETEC为产肠毒素性大肠埃希菌; EIEC为肠侵袭性大肠埃希菌; EAEC为肠集聚性大肠埃希菌

交叉现象则更显著。

依据标准, DEC的鉴定分类的步骤包括: 取新鲜粪便标本接种选择培养基培养, 再从平板上挑取可疑菌落作革兰染色镜检,生化试验并开展血清凝集以初步鉴定, 然后根据不同DEC类别开展毒素测定, 细胞黏附试验或者毒力基因等检测。由于标准中已有部分优势血清群/型可作为筛查依据, 同时多价血清覆盖范围较窄, 全套血清昂贵且难以获得, 这种依赖血清凝集的鉴定方式繁琐且低效, 漏检率极高, 但此种诊断方法目前仍被推荐并采用。本研究仅41.4%的DEC能明确O抗原型别, 24.6%能明确H抗原型别。EAEC、ETEC、EPEC的O血清凝集率分别为31.9%、70.6%和31.5%, 14株EHEC中仅2株凝集, 1株为O157:H7。曲梅等[1]对北京地区262株DEC研究发现仅有19株能明确O抗原型别; 深圳地区74株DEC中有63.5%能明确O抗原型别[2], 高翔等[3]也发现24株EPEC中仅2株能被血清凝集。本研究中696株经毒力基因检测方法鉴定为DEC菌株进行血清学分析, 仅75株能被分类, 其中33株毒力基因和血清学分类不一致。这些研究结果反映了各类DEC的血清区群/型可能发生变化, 或者有更多的血清区群/型类别的菌株存在, 因此单纯通过血清学筛选DEC, 会造成极大的漏检或误分。

本研究存在缺陷。尽管研究中采用的常见DEC诊断血清种类相对较多, 但还不够全面, 这在一定程度上影响了血清凝集率; 另外, 按照血清学鉴定方法, 对分离的大肠埃希菌直接进行毒力基因筛选, 再对阳性菌株进行血清学分析, 未同时依据标准直接采用多价血清开展初步鉴定, 因此对血清学鉴定效率的判断存在一定局限。

综上所述, 研究提示浙江地区DEC血清群/型种类多样, 单纯通过血清学筛选DEC会造成极大的漏检或误分, 建议采用分子生物学方法鉴定毒力基因的方式鉴定DEC, 必要时进行血清学筛查。


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