Determination of *Escherichia coli* O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia

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Abstract

*Escherichia coli* can be serotyped by determination of somatic (O), capsular (K), and flagellar (H) antigens, and clear associations exist between specific O antigens and pathogenic behavior. However, *E. coli* is very challenging to O type with traditional serologic methods, making new methods for *E. coli* somatic antigen detection highly desirable. Here, we describe a simple alternative molecular method for determination of the *E. coli* O type based on allele-specific polymerase chain reaction amplification of the 5′ portion of the *rfb* locus. We present our application of this new method to the detection of the 12 principal O types (O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157) found among bloodstream isolates of *E. coli*. This method allowed us to determine the O types of 153 strains previously typed using reference methods with an accuracy exceeding 90%. Moreover, some rough or nonagglutinating strains can be successfully typed.

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Keywords: *Escherichia coli*; Serotype O; Allele specific PCR; Bloodstream isolates

1. Introduction

*Escherichia coli* is a normal inhabitant of the intestine of most warm-blooded animals as well as humans. Some *E. coli* strains can cause a wide variety of intestinal and extraintestinal diseases (Donnenberg, 2002). *E. coli* can be serotyped by determination of somatic (O), capsular (K), and flagellar (H) antigens (Kauffmann, 1947), and some relationship exists between specific O types and pathogenic behavior (Orskov and Orskov, 1992). Because of these distinctive clinical associations and the strain discrimination O typing provides, O antigen determination of clinical *E. coli* isolates is a crucial modality for diagnostic and epidemiologic purposes. It could also be useful in the development of *E. coli* vaccines.

Unfortunately, *E. coli* is challenging to O type with the traditional method using antisera. This technique is complex, costly, and time consuming. Moreover, cross-reactivity of antisera with multiple O antigens occurs frequently. In addition, transition from the smooth (S) to rough (R) form, which is the result of mutations in 1 or more of the multiple genes controlling O antigen synthesis, renders isolates unable to produce O antigen and, therefore, refractory to typing. Genes controlling O antigen synthesis are in a region of 4.2 to 20 kb, termed the *rfb* cluster, which is generally bordered by the *gnd* and *galF* genes (Fig. 1). The number of genes in the *rfb* cluster varies from 6 to 19, and strains of different serotypes can show completely different gene sets and/or a different organization of conserved genes. O type-specific single nucleotide polymorphisms (SNPs) also exist within the conserved genes among several serotypes. Thus, based on the complete sequence of several O antigen gene clusters, polymerase chain reaction (PCR) assays using serotype gene-specific primers have been developed to determine some O types (Beutin et al., 2005a, 2005b; DebRoy et al., 2005; D’Souza et al., 2002, 2005; Feng et al., 2004; Fratamico et al., 2003; Paton and Paton, 1998, 1999; Perelle et al., 2002, 2004; Wang and Reeves, 1998; Wang et al., 1998; Wang et al., 2001, Wang et al., 2002). Alternatively, Coimbra et al. (2000) proposed...
an exhaustive approach for molecular serotype determination by using long-range PCR to amplify the entire rfb locus, followed by enzymatic restriction (rfb-RFLP). However, this method is time consuming, and the results are difficult to interpret because of the large number of generated bands.

Available sequences show that, within the rfb cluster, the gene located closest to gnd is often different in strains of different O types and suggest that the physical link between these 2 genes (i.e., gnd and the adjacent rfb gene) could be characteristic of the particular O type. Moreover, when the same gene is found close to gnd in several O types, sequence analysis suggests that certain nucleotide polymorphisms could allow differentiation between O types (i.e., O6 versus O7 or O26 versus O4 versus O172 versus O25) (Clermont, unpublished data). By taking advantage of these features, we developed a rapid and simple molecular technique for determining simultaneously several E. coli O types based on specific multiplex PCR amplifications between the 5′ portion of the rfb locus and gnd. This method was applied to the principal O types found in human bloodstream isolates of E. coli (Johnson and Stell, 2000; McCabe et al., 1978; Maslow et al., 1995) and was compared with the reference serologic method.

2. Materials and methods

2.1. Selection of studied O types

To develop our method, we focused on the O types most frequently observed in septicemia. Strains causing septicemia are part of the extraintestinal pathogenic E. coli (ExPEC) strains (Russo and Johnson, 2000). According to 3 large epidemiologic studies (Johnson and Stell, 2000; McCabe et al., 1978; Maslow et al., 1995), human bloodstream isolates belong mainly to the 12 following O types: O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157. These O types represent 75% to 81% of the typeable strains (Johnson and Stell, 2000; McCabe et al., 1978; Maslow et al., 1995).

2.2. Bacterial strains

The 370 strains used in this study correspond to reference strains or belong to various clinical collections. They included i) the E. coli reference (ECOR) collection (Amor et al., 2000; Ochman and Selander, 1984) (72 commensal and extraintestinal pathogenic strains); ii) 27 commensal or pathogenic strains, including reference strains, from Escobar-Paramo et al. (2004); iii) 55 strains causing extraintestinal infections from Picard et al. (1993); iv) 67 blood isolates from patients with urosepsis (Johnson and Stell, 2000); v) 140 bacteremia, pyelonephritis, and cystitis isolates from veterans (Sannes et al., 2004) or women (Johnson et al., 2005); vi) 7 archetypal strains from patients with urinary tract infection (J96, AD110, 536, F11, F63) or neonatal meningitis (C5, IHE3034); vii) the nonpathogenic strain Nissle 1917 (DSM6601) (Grozdanov et al., 2004); and viii) the laboratory strain K12-MG1655 (Blattner et al., 1997). All these strains have been previously serologically O typed at the State Serum Institute, Copenhagen, Denmark; the Laboratory Services, University of Guelph, Ontario, Canada; and/or the Gastroenteric Disease Center, Pennsylvania State University, University Park, PA.

The strains were used in a 2-step approach. In the 1st step, 24 strains (2 strains per O type) were used to design the primers and set up the assay (derivation set). The 2...
Table 1
List of the strains (derivation set) used for establishing the PCR-based O typing assay

<table>
<thead>
<tr>
<th>O type</th>
<th>Strain</th>
<th>Phylogenetic group</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>JGM4</td>
<td>B2</td>
<td>Picard et al., 1993</td>
</tr>
<tr>
<td></td>
<td>ECOR35</td>
<td>D</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td>O2</td>
<td>ECOR62</td>
<td>B2</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td></td>
<td>ECOR49</td>
<td>D</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td>O4</td>
<td>J96</td>
<td>B2</td>
<td>Blum et al., 1995</td>
</tr>
<tr>
<td></td>
<td>ECOR66</td>
<td>B2</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td>O6</td>
<td>EDL1493</td>
<td>A</td>
<td>Tomieporth et al., 1995</td>
</tr>
<tr>
<td></td>
<td>CFT073</td>
<td>B2</td>
<td>Welch et al., 2002</td>
</tr>
<tr>
<td>O7</td>
<td>ECOR38</td>
<td>D</td>
<td>Ochman and Selander, 1984</td>
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<td></td>
<td>ECOR39</td>
<td>D</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td>O12</td>
<td>Py190</td>
<td>A</td>
<td>Johnson et al., 2005</td>
</tr>
<tr>
<td></td>
<td>PUTI458</td>
<td>A</td>
<td>Johnson et al., 2005</td>
</tr>
<tr>
<td>O15</td>
<td>2H17</td>
<td>D</td>
<td>Blattner et al., 1997</td>
</tr>
<tr>
<td></td>
<td>ECOR24</td>
<td>A</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td>O16</td>
<td>K12-M1655</td>
<td>A</td>
<td>Blattner et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Py5</td>
<td>B2</td>
<td>Johnson et al., 2005</td>
</tr>
<tr>
<td>O18</td>
<td>RS218</td>
<td>B2</td>
<td>Silver et al., 1980</td>
</tr>
<tr>
<td></td>
<td>Py12</td>
<td>B2</td>
<td>Johnson et al., 2005</td>
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<td>O25</td>
<td>E2539-C1</td>
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<td>Tomieporth et al., 1995</td>
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<td></td>
<td>ECOR51</td>
<td>B2</td>
<td>Ochman and Selander, 1984</td>
</tr>
<tr>
<td>O75</td>
<td>C1845</td>
<td>B2</td>
<td>Bilge et al., 1989</td>
</tr>
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<td>ECOR64</td>
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</tr>
<tr>
<td>O157</td>
<td>EDL933</td>
<td>E</td>
<td>Pema et al., 2001</td>
</tr>
<tr>
<td></td>
<td>PUTI288</td>
<td>A</td>
<td>Johnson et al., 2005</td>
</tr>
</tbody>
</table>

*a Phylogenetic groups were assigned by the triplex method of Clermont et al. (2000) or by the multilocus sequence typing as in Escobar-Paramo et al. (2004).

representatives of each O type, when possible, were selected as belonging to different phylogenetic groups to obtain greater diversity (Table 1). In the 2nd step, 346 strains were used for validation of the assay (validation set). This latter set included both positive controls (129 strains with 1 of the target O types, including O1 [13 strains], O2 [24 strains], O4 [11 strains], O6 [16 strains], O7 [12 strains], O12 [5 strains], O15 [6 strains], O16 [10 strains], O18 [9 strains], O25 [7 strains], O75 [8 strains], and O157 [8 strains]) and negative controls (167 strains with a nontargeted O type [1–3 strains per serotype], including O3, O8–O11, O13, O17, O19, O21–O23, O26, O27, O29, O37, O39, O42, O44, O45, O48, O54, O55, O68, O71, O73, O77–O79, O82, O83, O86–O89, O91, O92, O102–O104, O106, O111–O113, O116, O117, O120, O121, O123, O126–O128, O132, O141, O143–O145, O147, O148, O150, O166, O168, and O173). This latter set also included 50 strains that were "rough", "O negative", or "nonreactive".

The phylogenetic group of origin (A, B1, B2, D, or E), as determined by the method of Clermont et al. (2000) or by multilocus sequence typing (Escobar-Paramo et al., 2004), was available from the literature for all the strains, except for the 55 strains from the Picard et al. collection, which have been typed in this work by the triplex PCR of Clermont et al. (2000). Bacteria were stored at −80 °C with glycerol and grown at 37 °C in Luria–Bertani (LB) broth or on LB agar plates.

2.3. rfb cluster amplification

Long-range PCR was performed with the Expand Long Template PCR system (Roche, Meylan, France). It was used to amplify the DNA region between gnd gene and JUMPstart (just upstream of many polysaccharide-associated gene start) sequence, a highly conserved 39-bp element (Hobbs and Reeves, 1994) (Fig. 1), by using modified primers previously published (Wang and Reeves, 1998) (Table 2). The PCR reaction was carried out according to the manufacturer’s instructions in a 30-µL volume, with 3 µL of buffer 3, 12 pmol of each primer, 500 µmol/L of each dNTP, 2.5 U of Taq mix, and 200 ng of genomic DNA. PCR was performed with an Eppendorf Mastercycler with MicroAm tubes (Eppendorf France; Le Pecq, France) in the following conditions: denaturation for 5 min at 94 ºC, 30 cycles of 10 s at 93 ºC, and 15 min at 63 ºC, with an increase of the step at 63 ºC by 20 s after cycle 10, and a final extension step of 7 min at 68 ºC.

2.4. Sequencing of the 5’ extremity of the rfb cluster

Long-range PCR fragments between gnd and JUMPstart of 2 strains belonging to each of the following O types—O1,
O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157—were sequenced with primer gndbis.f (Table 2 and Fig. 1) using the BigDye terminator cycle sequencing kit and an ABI 3100 Capillary DNA Sequencer (Applied Biosystem, Courtaboeuf, France). This provided interpretable sequences from 300 to 800 bp that allowed us to design O-specific reverse primers.

2.5. O-specific reverse primer choice

O-specific reverse primers were designed to obtain different PCR size fragments with the forward gndbis.f primer. They were designed by comparing available sequences (for serotypes O4 [strain 0544, accession no. AY568960], O6 [strains DSM6601, accession no. AJ426045, and CFT073, accession no. AE016766], O7 [strain VW187, accession no. AF125322], O15 [strain G1201, accession no. AY647261], O16 [strain K-12 MG1655, accession no. U00096], and O174 [strains C664-1992, accession no. AF061251, and EDL933, accession no. NC002655]) and sequences determined de novo by sequencing of the 5’ extremity of the rfb cluster as described above (Table 2 and Fig. 1). Furthermore, the absence of amplification by our primers was tested in silico on the other available rfb sequences (types O52, O59, O114, O138, O139, O149, O155, O172, and O174).

2.6. Multiplex PCR amplification

PCR reaction was carried out in a 20-μL volume containing 2 μL of 10× buffer (supplied with Taq polymerase), 20 pmol of each primer, 2 μmol/L of each dNTP, 1 U of Taq polymerase (ATGC Biotechnologie, Marne-la-Vallée, France), and 3 μL of bacterial lysate (3–5 colonies boiled in distilled water for 10 min, quenched in ice, and centrifuged). PCR was performed with an Eppendorf Mastercycler with MicroAm tubes in the following conditions: denaturation for 4 min at 94 °C, 30 cycles of 5 s at 94 °C and 10 s at 59 °C, and a final extension step of 5 min at 72 °C. Primer sets 1 and 2 each contained a single (consensus) forward primer and 6 (6 O specific) reverse primers. The primers of primer set 1 included gndbis.f, rfbO1.r, rfbO2a.r, rfbO18.r, rfbO16.r, rfbO6a.r, and rfbO7.r. The primers of primer set 2 included gndbis.f, rfbO4.r, rfbO12.r, rfbO25a.r, rfbO75.r, rfbO15.r, and rfbO157.r (Table 2). PCR products were loaded on 2% agarose gel with ethidium bromide. After electrophoresis, gels were photographed under ultraviolet (UV) light.

2.7. Interlaboratory standardization of the method

To provide validation of the O PCR assay and to explore its portability across laboratories, an independent laboratory (that of J.R.J.) established the assay after it was 1st developed in the E.D. laboratory. Minor assay modifications were required to achieve reliable amplification of all 12 O-specific PCR products, including individualized annealing temperatures for 2 primer pairs (O75, 58 °C; O4, 67 °C) and, particularly for the O1 primers, use of a specific thermal cycler (with ramping speeds of 1.2 °C/s). Twenty-four strains were tested in both laboratories. Of the 346 E. coli unique isolates studied, 246 and 100 were screened only in the E.D. or J.R.J. laboratory, respectively.

2.8. Long-range PCR and RFLP analysis

To resolve discrepancies encountered between conventional and PCR-based O typing results, we performed long-range PCR on the entire rfb locus with gnd.f and JUMPstart.r primers (Fig. 1 and Table 1), followed by MboII digestion and electrophoresis of the PCR products, as in Coimbra et al. (2000). The Coimbra et al. protocol was slightly modified as follows: 4 μL of long-range PCR product was mixed with 18 U of MboII enzyme (New England Biolabs, St Quentin-en-Yvelines, France) and supplied buffer in a final volume of 10 μL. After incubation at 37 °C for 2 h, tubes were heated at 72 °C for 10 min to irreversibly denature the MboII enzyme (Coimbra et al., 2000). The entire volume was then loaded on a 2% agarose gel with ethidium bromide. After electrophoresis, gels were photographed under UV light.

3. Results and discussion

3.1. rfb cluster 5′ extremity sequence comparison, selection of primers, and derivation set results

Twenty-four strains, representing the 12 O types of interest (2 strains per O type), were used to determine the sequence of the 5′ portion of the rfb locus (Table 1). The entire rfb locus of each strain was amplified by long-range PCR. As expected, PCR fragments of 5000 to 17000 bp were obtained, with the same length being obtained for different representatives of a particular O type even if the strains belonged to different phylogenetic groups (data not shown). When compared with the available GenBank sequences, no difference was evidenced in the sequences of the same O type except for the O75 strains, which differed by 1 nucleotide. Specific primers were then designed for particular type-specific genes (O types O1, O2, O16, O18, O75, and O157) or SNPs within conserved genes (O types O4, O6, O7, O12, O15, and O25) so as to yield PCR fragments with different lengths (ranging from 200 to 700 bp) after PCR amplification. Experimental validation was obtained from this derivation set of strains, the expected PCR product lengths being assessed after migration of PCR products (Figs. 2 and 3).

3.2. Reproducibility, sensitivity, and specificity of the method

Reproducibility was tested within and between laboratories. Repetitive analysis up to 12 times of the derivation set strains, as well as replicate typing of 24 common strains in the 2 laboratories, yielded identical results (data not shown). The assay proved to be portable and highly accurate in both
laboratories, but some optimization was required (see Materials and methods).

The sensitivity of the method was initially tested on 153 strains belonging to 1 of the 12 targeted O types. Overall, the sensitivity was 92%. A closer examination of the results according to the specific O types indicated a variable sensitivity rate of 71% to 100%, with the lower sensitivity being observed for O type O12 (71%), whereas a 100% sensitivity was obtained for O types O4, O15, O16, O18, O75, and O157 (Table 3). Two sorts of problems were encountered, that is, the PCR assay yielded either i) no PCR product (1 O6, 1 O25, and 4 O2 strains) or ii) a product corresponding with an O type other than that expected (1 O1, 1 O6, 2 O7, and 2 O12 strains; these appeared by the PCR assay as O18, O18, O6, and O16, respectively). In addition, the specificity of the method was tested on 167 strains representing 61 O types not targeted by our PCR approach. No false amplification was obtained with this set of 167 strains. Overall, the only 6 false-positive results obtained were for the 6 strains with targeted O types (as described above), which appeared falsely O6, O16, or O18 (Table 3).

For the 12 strains that exhibited discrepancies between standard and PCR-based O typing, long-range PCR, followed by RFLP analysis (Coimbra et al., 2000) and sequencing, was performed. Three sorts of discrepancies were elucidated. i) When strains failed to amplify with specific primers (1 O25, 1 O6, and 4 O2 strains), the long-range PCR product lengths were different from the typical one. For the O25 strain (ECOR15), long-range PCR RFLP showed a not-previously–reported pattern that was clearly distinct from the typical one (Fig. 4). Moreover, the long-range PCR sequencing showed an unknown sequence for the 5′ part of the rfb locus close to gnd. For the O6 strain, sequencing also showed an unknown sequence. For the O2 strains, 3 distinct long-range PCR product lengths were observed, as previously reported (Coimbra et al., 2000). The long-range PCR sequencing showed 2 unknown sequences and 1 corresponding to the 5′ part of the rfb locus close to gnd of an O114 strain (AY573377, Feng et al., 2004). Primer names rfbO25a, rfbO6a, and rfbO2a were used for these O types (see Materials and methods and Table 2) to identify the primers corresponding to the sequences of the reference strain of each O type. These data indicate that several rfb variants exhibiting major architectural changes can yield identical serologically detected O types. ii) Long-range PCR RFLPs were performed on the O1 and O6 strains that appeared O18 by our PCR-based typing assay, and on the 2 O7 strains that

<table>
<thead>
<tr>
<th>O type</th>
<th>No. of tested strains</th>
<th>Specific PCR-positive strains</th>
<th>Sensitivity (%)</th>
<th>Specificity (false positive/no. of tested strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>15</td>
<td>14</td>
<td>93</td>
<td>0/305</td>
</tr>
<tr>
<td>O2</td>
<td>26</td>
<td>22</td>
<td>85</td>
<td>0/294</td>
</tr>
<tr>
<td>O4</td>
<td>13</td>
<td>13</td>
<td>100</td>
<td>0/307</td>
</tr>
<tr>
<td>O6</td>
<td>18</td>
<td>16</td>
<td>89</td>
<td>2/302</td>
</tr>
<tr>
<td>O7</td>
<td>14</td>
<td>12</td>
<td>86</td>
<td>0/306</td>
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<tr>
<td>O12</td>
<td>7</td>
<td>5</td>
<td>71</td>
<td>0/313</td>
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<td>O15</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>0/312</td>
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<tr>
<td>O16</td>
<td>12</td>
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<td>100</td>
<td>2/308</td>
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<td>11</td>
<td>11</td>
<td>100</td>
<td>2/309</td>
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<td>O25</td>
<td>9</td>
<td>8</td>
<td>89</td>
<td>0/311</td>
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<tr>
<td>O75</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>0/310</td>
</tr>
<tr>
<td>O157</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>0/310</td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>141</td>
<td>92</td>
<td>6/320</td>
</tr>
</tbody>
</table>

* The denominator number for specificity varies among the 12 individual O types because it represents 320 minus the number of isolates serologically positive for the particular O type.
appeared O6. It showed, for the O7 and O6 strains, profiles more or less closely related, but not identical, to the expected ones based on their serologic O type (Fig. 4). For the O1 strain, the RFLP pattern did not correspond to the typical O1 or O18 ones (Fig. 4). This suggests that these strains harbored a rearranged rfb locus. Sequencing of the 5' portion of the rfb locus for the O1 and O6 strains showed a typical O18 sequence, explaining the fact that these strains appeared O18 with the PCR method. Sequences of the O7 strains showed that these strains harbor, as expected based on published sequence data for the O7 rfb region (Marolda and Valvalno, 1993), the manB gene close to the gnd gene, but with the sequence of Shigella sonnei, which contain both O7-like and O6-like SNPs, the latter being what we used for PCR O typing. iii) Lastly, long-range PCR RFLPs of the 2 O12 strains that appeared O16 by the PCR assay showed a typical O16 profile (Fig. 4). Moreover, sequencing of the 5' part of the rfb locus shows in both strains a typical O16 type sequence.

3.3. How to explain the observed genotype versus phenotype discrepancies?

Our PCR-based assay detects sequence diversity within regions possibly not directly related to O antigen expression, but presumably genetically linked to the responsible genes. For conventional serologic O typing, the targets are specific (undefined) epitopes within the polysaccharide portion of lipopolysaccharide. These are dependent on the presence of specific sugars, in specific stereochemical orientations, regardless of the nature of the responsible synthetic enzymes and their respective genes. It would not be altogether surprising if the same O antigen could arise via different synthetic pathways, or if the 5' end of the rfb regions was to exhibit some degeneracy even among strains that all synthesize the exact same polysaccharide, even via the same enzyme pathway (which could occur if the rfb 5' region is not directly involved in polysaccharide synthesis). Such scenarios conceivably could explain the first two types of discrepancies that we have observed and represent a limitation of our assay. On the other hand, the O12/O16 discrepancy comes probably from a cross-reactivity between these two O types according to conventional serologic O typing. The putative O12 isolates that were O16 by PCR exhibited random amplified polymorphic DNA (RAPD) genomic profiles and virulence profiles indistinguishable from those of the sole O16:K1:H6 isolate in the urosepsis collection (Johnson and Stell, 2000). This, plus the reactivity of certain O16:K1 isolates with both O12 and O16 antisera (J.R.J., unpublished data), and the finding that such strains give sequence and long-range PCR RFLP pattern identical to typical O16 strains (see Fig. 4 for an example with the strain CL22) suggested that the O PCR assay may have correctly identified these isolates as being O16.

3.4. The “rough”, “O-negative”, or “nonreactive” strains

Three of the 4 putative O-rough isolates within the urosepsis collection yielded an O-PCR product consistent with the isolate’s presumed clonal group assignment (O1:K1:H7, O4:K12:H5, or O6:K2:H1, respectively), as inferred by comparison with other isolates in the urosepsis collection according to K:H serotype, RAPD profile, and virulence profile (not shown). This suggested accurate detection of serologically unapparent O antigen-encoding sequences in these strains. (The 4th rough isolate OR:K1:H6 was O PCR negative.) Similarly, strain K12-MG1655 appears O16 with our assay, as was previously determined by the long-range PCR RFLP assay (Coimbra et al., 2000).

To further assess the ability of the O PCR assay to define O groups among serologically indeterminate isolates, the assay was next used to test 46 O-nontypeable isolates (as determined by the E. coli Reference Center, University Park, PA). The 46 isolates were selected specifically for their ambiguous O status among isolates from veterans with bacteremia, or women with acute pyelonephritis or cystitis, or from the ECOR collection. These 46 isolates putatively included 25 from phylogenetic groups B2 and 21 (collectively) from groups A, B1, and D, as defined by PCR (Clermont et al., 2000). Overall, 17 (37%) of the 46 O-indeterminate isolates yielded an O-specific PCR product (4 O75; 4 O6; 2 each of O2, O12, and O16; and 1 each of O1 and O7). Supporting the biologic validity of these results, O PCR positivity was significantly more frequent among isolates putatively from group B2, which encompasses mainly strains involved in extraintestinal infections, including sepsis, than those putatively from other phylogenetic groups (15/25 vs 2/21, P < .001, Fisher’s exact test).
4. Concluding remarks

In this work, we have developed a robust PCR method to rapidly determine the O type of E. coli strains. Using the variability of the 5’ portion of the rfb cluster, we have been able to design primers specific for O types O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157, allowing us to determine the O types of 153 strains (as previously typed using reference method) with an accuracy exceeding 90%. The observed discrepancies between the 2 methods were easily explained by the fact that the PCR assay assesses the phenotype, whereas the traditional reference method using antisera assesses the phenotype, indicating that there may not exist a perfect “gold standard” for O typing. In addition to the rapidity and simplicity of our PCR-based method, no special reagents or equipment are required, meaning that the assay can be used easily and inexpensively in any laboratory equipped for diagnostic PCR. Moreover, some rough or nonagglutinating strains can be successfully typed. This is particularly interesting because these strains sometimes constitute a large portion of studied strains, which can lead to bias or ambiguity in epidemiologic studies.

Finally, using our approach, primer sets conceivably could be designed to be locale and/or pathology specific (e.g., for newborn meningitis strains, O type O45 in France [Bonacorsi et al., 2003] or O83 in the Netherlands [Johnson et al., 2003]; for VETEC strains, O types O26, O55, O91, O103, O111, O113, O126, and/or O145, in addition to O157). This could lead to the development of “epidemiologic-specific primer kits” for O typing.

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References


