Comparison of pulsed-field gel electrophoresis & repetitive sequence-based PCR methods for molecular epidemiological studies of *Escherichia coli* clinical isolates

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**Background & objectives:** PFGE, rep-PCR, and MLST are widely used to identify related bacterial isolates and determine epidemiologic associations during outbreaks. This study was performed to compare the ability of repetitive sequence-based PCR (rep-PCR) and pulsed-field gel electrophoresis (PFGE) to determine the genetic relationships among *Escherichia coli* isolates assigned to various sequence types (STs) by two multilocus sequence typing (MLST) schemes.

**Methods:** A total of 41 extended-spectrum β-lactamase- (ESBL-) and/or AmpC β-lactamase-producing *E. coli* clinical isolates were included in this study. MLST experiments were performed following the Achtman’s MLST scheme and the Whittam’s MLST scheme, respectively. Rep-PCR experiments were performed using the DiversiLab system. PFGE experiments were also performed.

**Results:** A comparison of the two MLST methods demonstrated that these two schemes yielded compatible results. PFGE correctly segregated *E. coli* isolates belonging to different STs as different types, but did not group *E. coli* isolates belonging to the same ST in the same group. Rep-PCR accurately grouped *E. coli* isolates belonging to the same ST together, but this method demonstrated limited ability to discriminate between *E. coli* isolates belonging to different STs.

**Interpretation & conclusions:** These results suggest that PFGE would be more effective when investigating outbreaks in a limited space, such as a specialty hospital or an intensive care unit, whereas rep-PCR should be used for nationwide or worldwide epidemiology studies.

**Key words** *Escherichia coli* - genetic relationship - MLST - PFGE - rep-PCR - sequence type
Pulsed-field gel electrophoresis (PFGE), repetitive extragenic palindromic PCR (rep-PCR), and multilocus sequence typing (MLST) are widely used to identify related bacterial isolates and determine epidemiologic associations during outbreaks\textsuperscript{5,6}. Among these molecular methods, PFGE is generally considered the gold standard because of its high discriminatory ability, and is, therefore, used routinely to subtype many different bacterial species including \textit{Escherichia coli}. However, PFGE is a laborious, time-consuming procedure that needs to be performed by a well-trained technician\textsuperscript{6}. Furthermore, if there is degradation of genomic DNA during plug preparation, banding patterns may not be observed. Brolund et al\textsuperscript{6} reported that PFGE band patterns were interpretable in only 88 per cent of \textit{E. coli} isolates. Another major limitation of PFGE is its low inter-laboratory reproducibility.

Recently, rep-PCR using the DiversiLab system (bioMérieux, Grenoble, France) has been used to characterize the genomic relatedness of bacterial isolates\textsuperscript{5,6}. This technique requires less time to perform than PFGE, is technically simple, and has been shown to have similar discriminative power to PFGE\textsuperscript{7}.

In this study, the ability of rep-PCR and PFGE methods was compared to determine the genetic relationships among \textit{E. coli} isolates assigned to various sequence types (STs) by two MLST schemes, namely Achtman’s scheme (www.mlst.ucc.ie) and Whittam’s scheme (www.shigatox.net). The two MLST schemes were further studies to determine whether these yielded compatible results.

**Material & Methods**

This study was performed in the Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Republic of Korea.

**Bacterial strains:** A total of 41 extended-spectrum β-lactamase- (ESBL-) and/or AmpC β-lactamase-producing \textit{E. coli} clinical isolates were evaluated in this study. Of these isolates, 34 harboured ESBL, four harboured AmpC β-lactamase, and three harboured both ESBL and AmpC β-lactamase (Table). The isolates were identified using either a Vetek 2 GN card (bioMérieux, France) or by 16S rRNA sequencing. Detection of genes coding for plasmid-borne ESBLs and AmpC β-lactamases was performed by PCR amplification as described previously\textsuperscript{8,9}. The PCR products were directly sequenced using an automated DNA sequencer (Applied Biosystems model 3730xl, Weiterstadt, Germany).

**MLST by Achtman’s MLST scheme:** The \textit{E. coli} isolates were typed by amplifying partial fragments of seven housekeeping genes (\textit{adk}, \textit{funC}, \textit{gyrB}, \textit{icd}, \textit{mdh}, \textit{purA}, and \textit{recA}) following the protocols available at the website: http://mlst.ucc.ie/mlst/dbs/Ecoli.

**MLST by Whittam’s MLST scheme:** The \textit{E. coli} isolates were also typed by amplifying partial fragments of seven housekeeping genes (\textit{aspC}, \textit{clpX}, \textit{fadD}, \textit{icaA}, \textit{lpsP}, \textit{mdh}, and \textit{uidA}) following the protocols available at the website: http://www.shigatox.net/ecmlst.

**PFGE:** Plugs containing whole genomic DNA of the \textit{E. coli} isolates were digested with the \textit{XbaI} restriction enzyme. DNA fragments were separated by PFGE using a CHEF-DRII device (Bio-Rad, USA). The conditions used for \textit{XbaI}-macrorestriction analysis by PFGE were 6 V/cm for 20 h with pulse times ranging from 0.5 to 60 sec at 14°C. A lambda ladder (Bio-Rad) was used as a DNA size marker. Gel images in TIFF format were exported to Molecular Analyst Fingerprinting Software Ver. 3.2 (Bio-Rad) for analysis. \textit{E. coli} isolates were compared using the band-based dice coefficient. Dendrograms were generated by the unweighted pair group method with arithmetic averages and a 1.0 per cent position tolerance, and DNA relatedness was calculated based on the criteria described by Tenover et al\textsuperscript{10}. Isolates showing >80 per cent similarity were considered to be related.

**Rep-PCR:** Rep-PCR experiments were performed using the DiversiLab system (bioMérieux, Grenoble, France) according to the manufacturer’s instructions. Results were analyzed with DiversiLab software using the Kullback-Leibler method to determine distance matrices and with the unweighted pair group method with arithmetic averages to create a dendrogram. Isolates showing >95 per cent similarity were considered to be related.

**Results**

**Achtman’s scheme vs. Whittam’s scheme:** Of the 22 \textit{E. coli} isolates identified as ST131 using Achtman’s MLST scheme, 16 were identified as ST34, one each as the single locus variants ST872 and ST876, and four as the double-locus variants ST856 (n = 3) and ST873 (n = 1) using Whittam’s MLST scheme. The four \textit{E. coli} isolates identified as ST171 by Whittam’s MLST scheme were identified as ST10 (n = 1), its single-locus variants ST44 (n = 1) and ST744 (n = 1), and its double-locus variant ST617 (n = 1) by Achtman’s MLST scheme. In addition, two \textit{E. coli} isolates identified as ST857 using Whittam’s MLST...
Table. Sequence types of *E. coli* clinical isolates according to Achtman’s and Whittam’s MLST schemes

| Isolate   | Specimen type | MLST | β-lactamase gene |
|-----------|---------------|------|------------------|
|           |               | Achtman’s scheme | Whittam’s scheme |                     |
|           |               | ST  | adK | fumC | gyrB | icd | mdh | purA | recA | ST  | aspC | clpX | fadD | icdA | lysP | mdh | uidA |
| B060236   | Blood         | 131 | 53  | 40   | 47   | 36  | 28  | 29   |      | 34  | 21   | 53   | 56   | 8    | 40   | 55   | 72   |
| E07294    | Blood         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| E07348    | Blood         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| E08230    | Blood         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| E08344    | Blood         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| E09069    | Blood         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| A05       | Stool         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| H04       | Stool         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| H05       | Stool         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| H08       | Stool         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| H09       | Stool         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| H24       | Stool         | 131  | 53  | 40   | 47   | 36  | 28  | 29   |      | 856 | 21   | 53   | 215  | 8    | 40   | 55   | 55   |
| H30       | Stool         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| BDE0502   | Urine         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| BDE0516   | Body fluid    |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| WKE0506   | pus           |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| E08420    | Blood         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| HYE0515   | Urine         |      |     |      |      |     |     |      |      |     |      |      |      |      |      |      |      |
| E09071    | Blood         | 872  | 21  | 71   | 56   | 8    | 40  | 55   | 72   |      |      |      |      |      |      |      |      |
| E09108    | Blood         | 873  | 21  | 10   | 215  | 8    | 40  | 55   | 72   |      |      |      |      |      |      |      |      |
| E06368    | Blood         | 876  | 21  | 53   | 8    | 40  | 55   | 72   |      |      |      |      |      |      |      |      |      |
| B090371   | Blood         | 10   | 10  | 11   | 4    | 8   | 8   | 8    | 2    |      |      |      |      |      |      |      |      |      |
| B081373   | Blood         | 38   | 4   | 26   | 2    | 25  | 5   | 5    | 19   |      |      |      |      |      |      |      |      |      |
| E08013    | Blood         | 44   | 10  | 11   | 4    | 8   | 8   | 8    | 7    |      |      |      |      |      |      |      |      |      |
| E08342    | Blood         | 46   | 8   | 7    | 1    | 8   | 8   | 8    | 6    |      |      |      |      |      |      |      |      |      |
| E08414    | Blood         | 95   | 37  | 38   | 19   | 37  | 17  | 11   | 26   |      |      |      |      |      |      |      |      |      |
| bAE et al.: COMPARISON OF PFGE & REP-PCK FOR GENETIC STUDIES IN E. COI }
| Isolate | Specimen type | ST  | adK | fumC | gyrB | icd | mdh | purA | recA | ST  | aspC | clpX | fadD | icdA | lysP | mdh | uidA | MLST | β-lactamase gene |
|---------|---------------|-----|-----|------|------|-----|-----|------|------|-----|------|------|------|------|------|-----|------|-------|-----------------|
| H20     | Blood         | 95  | 37  | 38   | 19   | 37  | 17  | 11   | 26   | 29  | 21   | 24   | 10   | 8    | 17   | 11  | 25   | MLST   | βCTX-M-57        |
| E08284  | Blood         | 112 | 13  | 44   | 9    | 22  | 16  | 30   | 34   | 875 | 21   | 7    | 10   | 132  | 17   | 36  | 134  | MLST   | βCTX-M-15        |
| E07409  | Blood         | 354 | 85  | 88   | 78   | 29  | 59  | 58   | 62   | 857 | 72   | 36   | 216  | 85   | 118  | 234 |       | MLST   | βCTX-M-14        |
| E08132  | Blood         | 393 | 18  | 106  | 17   | 6   | 5   | 5    | 4    | 39  | 25   | 29   | 55   | 59   | 39   | 33  | 74   | MLST   | βCTX-M-14        |
| SCE0503 | Sputum        | 405 | 35  | 37   | 29   | 25  | 4   | 5    | 73   | 288 | 75   | 74   | 84   | 73   | 29   | 69  | 43   | MLST   | βCTX-M-14        |
| E05051  | Blood         | 410 | 6   | 4    | 12   | 1   | 20  | 18   | 7    | 88  | 32   | 12   | 61   | 12   | 1    | 12  | 12   | MLST   | βCTX-M-15        |
| E08331  | Blood         | 457 | 101 | 88   | 97   | 108 | 26  | 79   | 2    | 867 | 72   | 177  | 218  | 44   | 46   | 80  | 238  | MLST   | βCTX-M-14        |
| E07483  | Blood         | 617 | 10  | 11   | 4    | 8   | 8   | 13   | 73   | 171 | 3    | 3    | 1    | 1    | 1    | 1   | 1    | MLST   | βCTX-M-15        |
| E09060  | Blood         | 648 | 92  | 4    | 87   | 96  | 70  | 58   | 2    | 871 | 61   | 10   | 54   | 63   | 46   | 62  | 12   | MLST   | βCTX-M-15        |
| H21     | Urine         | 744 | 10  | 11   | 135  | 8   | 8   | 8    | 2    | 171 | 3    | 3    | 1    | 1    | 1    | 1   | 1    | MLST   | βSV2            |
| E08248  | Blood         | 773 | 6   | 165  | 4    | 10  | 7   | 8    | 6    | 85  | 3    | 3    | 2    | 4    | 6    | 2   | 36   | MLST   | βCTX-M-14        |
| E08437  | Blood         | 964 | 35  | 183  | 29   | 25  | 4   | 5    | 73   | 288 | 75   | 74   | 84   | 73   | 29   | 69  | 69   | MLST   | βCTX-M-15        |
| B061647 | Blood         | 1011| 6   | 4    | 159  | 44  | 112 | 1    | 17   | 69  | 119  | 132  | 29   | 77   | 18   | 124| 102  | MLST   | βCTX-M-15        |
| E08398  | Blood         | 2037| 85  | 88   | 78   | 37  | 59  | 58   | 62   | 857 | 72   | 36   | 216  | 85   | 118  | 234 |       | MLST   | βCTX-M-14        |
scheme were identified as ST354 and its single-locus variant ST2037 by Achtman’s MLST scheme (Table). These results indicated that the two schemes yielded compatible results.

**MLST vs. rep-PCR:** The 22 *E. coli* isolates identified as ST131 using Achtman’s MLST scheme were >95 per cent similar by rep-PCR (Fig. 1A). However, the five isolates of ST38, ST46, ST95, ST354, and ST648 also showed >95 per cent similarity to the ST131 isolates (I in Fig. 1B). Furthermore, two isolates typed as different ST isolates using Achtman’s MLST scheme, ST44 and ST393, were also >95 per cent similar to one another (II in Fig. 1B). The remaining ST isolates showed <95 per cent similarity to each other (Fig. 1B). These results demonstrate that rep-PCR can accurately group *E. coli* isolates belonging to the same ST together, but this method demonstrated limited ability to discriminate between *E. coli* isolates belonging to different STs.

**PFGE vs. MLST:** In the *Xba*I-macrorestriction experiments, of the 22 *E. coli* isolates identified as ST131 by Achtman’s MLST scheme, most of the isolates showed <80 per cent similarity (Fig. 2A), except for a few isolates (I-IV in Fig. 2A) that showed >80 per cent similarity. However, most of the isolates classified as belonging to different STs by MLST showed <80 per cent similarity by PFGE (Fig. 2B), although there were some isolates (I and II in Fig. 2B) belonging to different STs that showed >80 per cent similarity. These results indicate that PFGE can segregate *E. coli* isolates belonging to different STs into different types, but is not optimal for grouping *E. coli* isolates of the same ST.

**Discussion**

Achtman’s MLST scheme is based on seven housekeeping genes (*adk, fumC, gyrB, icdA, mdh, purA,* and *recA*) and has been used in epidemiological studies of antimicrobial resistance, whereas Whittam’s MLST scheme, which is also based on a set of seven housekeeping genes (*aspC, clpX, fadD, icdA, lysP, mdh,* and *uidA*), has been used mainly in the field of food microbiology. *E. coli* isolates harbouring the same antimicrobial resistance genes have been detected in food, the gastrointestinal tract of food-producing and companion animals, and in humans. In other words, food-producing and companion animals may play a significant role in the dissemination of antibiotic resistant bacteria in humans. Therefore, comparing the epidemiology of bacteria isolated from humans and food-producing animals or food is crucial to understand the mechanisms and components

![Fig. 1. Banding patterns of 22 E. coli isolates identified as ST131 using Achtman’s MLST scheme (A) and 20 E. coli isolates identified as non-ST131 according to Achtman’s MLST scheme, (B) obtained by rep-PCR. The dotted line in Fig. 1B indicates 95 per cent similarity.](image-url)
underlying the dissemination of antimicrobial resistance genes. Unfortunately, using different MLST schemes to subtype *E. coli* isolates from humans and food-producing animals complicated epidemiological comparisons.

This study showed that the two schemes yielded compatible results, even though employing different loci. These results could be used in the understanding of reports for *E. coli* epidemiology. For example, six enterotoxigenic *E. coli* isolates identified as ST34 by Whittam’s MLST scheme in a study by Steinsland et al.\(^\text{13}\) would be considered ST131 *E. coli* isolates according to Achtman’s MLST scheme. And CTX-M-14 extended-spectrum β-lactamase-producing *E. coli* isolates from Spain identified as ST10 by Achtman’s MLST scheme in a study by Valverde et al.\(^\text{14}\) would be considered ST171 *E. coli* isolates according to Whittam’s MLST scheme.

PFGE showed higher discriminative power than rep-PCR in grouping *E. coli* isolates belonging to different STs, whereas rep-PCR accurately grouped *E. coli* isolates belonging to the same ST together, but PFGE did not. This study showed that the appropriate molecular typing method for an epidemiological study should be chosen based on the particular characteristics of that study. PFGE is more suited for investigating outbreaks in a limited space such as a specialty hospital or an intensive care unit due to its high discriminatory abilities, whereas rep-PCR should be used in nationwide or worldwide epidemiology studies because it is more efficient at grouping strains belonging to the same STs together.

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