Indian J Med Res 140, November 2014, pp 679-685

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

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Received March 26, 2013

***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[1-4]. 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 *Escherichia coli*. However, PFGE is a laborious, time-consuming procedure that needs to be performed by a well-trained technician[5]. Furthermore, if there is degradation of genomic DNA during plug preparation, banding patterns may not be observed. Brolund *et al*[5] reported that PFGE band patterns were interpretable in only 88 per cent of *E. coli* isolates. Another major limitation of PFGE is its low inter-laboratory reproducibility.

Recently, rep-PCR using the DiversiLab system (bioMerieux, Grenoble, France) has been used to characterize the genomic relatedness of bacterial isolates[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[7].

In this study, the ability of rep-PCR and PFGE methods was compared to determine the genetic relationships among *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 *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 Vitek 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[8,9]. The PCR products were directly sequenced using an automated DNA sequencer (Applied Biosystems model 3730*xl*, Weiterstadt, Germany).

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

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

PFGE: Plugs containing whole genomic DNA of the *E. coli* isolates were digested with the *Xba*I restriction enzyme. DNA fragments were separated by PFGE using a CHEF-DRII device (Bio-Rad, USA). The conditions used for *Xba*I-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. *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*[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[7].

Results

Achtman's scheme vs. Whittam's scheme: Of the 22 *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 *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 *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																	$bla_{CTX-M-15}$
E07294	Blood																	$bla_{CTX-M-15}$
E07348	Blood																	$bla_{CTX-M-15}$
E08230	Blood																	$bla_{CTX-M-14}$
E08344	Blood																	$bla_{CTX-M-14}$
E09069	Blood																	$bla_{CTX-M-14}$
A05	Stool																	bla_{CMY-2}
H04	Stool									34	21	53	56	8	40	55	72	$bla_{CTX-M-14}$
H05	Stool																	$bla_{CTX-M-14}$
H08	Stool																	bla_{CMY-2}
H09	Stool	131	53	40	47	13	36	28	29									$bla_{CTX-M-15}$
H24	Stool																	$bla_{CTX-M-15}$, bla_{CMY-2}
H30	Stool																	bla_{CMY-2}
BDE0502	Urine																	bla_{SHV-2a}, $bla_{CTX-M-14}$, $bla_{CTX-M-15}$
BDE0516	Body fluid																	$bla_{CTX-M-14}$
WKE0506	pus																	$bla_{CTX-M-14}$
E08032	Blood																	$bla_{CTX-M-14}$
E08420	Blood									856	21	53	215	8	40	55	55	$bla_{CTX-M-14}$
HYE0515	Urine																	$bla_{CTX-M-14}$
E09071	Blood									872	21	71	56	8	40	55	72	$bla_{CTX-M-15}$
E09108	Blood									873	21	10	215	8	40	55	72	$bla_{CTX-M-14}$, bla_{CMY-2}
E06368	Blood									876	21	53	10	8	40	55	72	bla_{SHV-12}, bla_{DHA-1}
B090371	Blood	10	10	11	4	8	8	8	2	171	3	3	1	1	1	1	1	$bla_{CTX-M-14}$
B081373	Blood	38	4	26	2	25	5	5	19	303	72	71	83	73	53	68	101	$bla_{CTX-M-14}$
E08013	Blood	44	10	11	4	8	8	8	7	171	3	3	1	1	1	1	1	$bla_{CTX-M-15}$
E08342	Blood	46	8	7	1	8	8	8	6	738	4	77	13	1	1	1	134	$bla_{CTX-M-15}$
E08414	Blood	95	37	38	19	37	17	11	26	29	21	24	10	8	17	11	25	$bla_{CTX-M-15}$

Contd...

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*	
H20	Blood	95	37	38	19	37	17	11	26	29	21	24	10	8	17	11	25	$bla_{\text{CTX-M-57}}$
E08284	Blood	112	13	44	9	22	16	30	34	875	21	7	10	132	17	36	134	$bla_{\text{CTX-M-15}}$
E07409	Blood	354	85	88	78	29	59	58	62	857	72	36	216	85	118	178	234	$bla_{\text{CTX-M-14}}$
E08132	Blood	393	18	106	17	6	5	5	4	39	25	29	55	59	39	33	74	$bla_{\text{CTX-M-14}}$
SCE0503	Sputum	405	35	37	29	25	4	5	73	288	75	74	84	73	29	69	43	$bla_{\text{CTX-M-14}}$
E05051	Blood	410	6	4	12	1	20	18	7	88	32	12	61	12	1	12	12	$bla_{\text{CTX-M-15}}$
E08331	Blood	457	101	88	97	108	26	79	2	867	72	177	218	44	46	80	238	$bla_{\text{CTX-M-14}}$
E07483	Blood	617	10	11	4	8	8	13	73	171	3	3	1	1	1	1	1	$bla_{\text{CTX-M-15}}$
E09060	Blood	648	92	4	87	96	70	58	2	871	61	10	54	63	46	62	12	$bla_{\text{CTX-M-15}}$
H21	Urine	744	10	11	135	8	8	8	2	171	3	3	1	1	1	1	1	$bla_{\text{CMY-2}}$
E08248	Blood	773	6	165	4	10	7	8	6	85	3	3	2	4	6	2	36	$bla_{\text{CTX-M-14}}$
E08437	Blood	964	35	183	29	25	4	5	73	288	75	74	84	73	29	69	69	$bla_{\text{CTX-M-15}}$
B061647	Blood	1011	6	4	159	44	112	1	17	69	119	132	29	77	18	124	102	$bla_{\text{CTX-M-15}}$
E08398	Blood	2037	85	88	78	37	59	58	62	857	72	36	216	85	118	178	234	$bla_{\text{CTX-M-14}}$

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[11]. In other words, food-producing and companion animals may play a significant role in the dissemination of antibiotic resistant bacteria in humans[12]. Therefore, comparing the epidemiology of bacteria isolated from humans and food-producing animals or food is crucial to understand the mechanisms and components

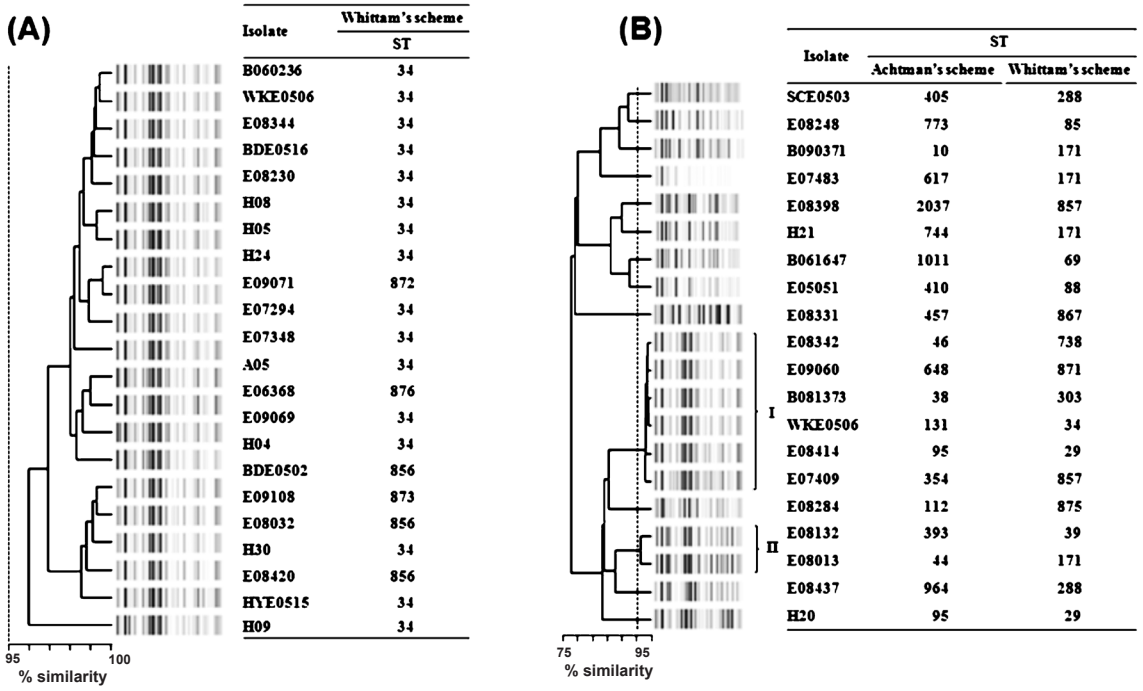
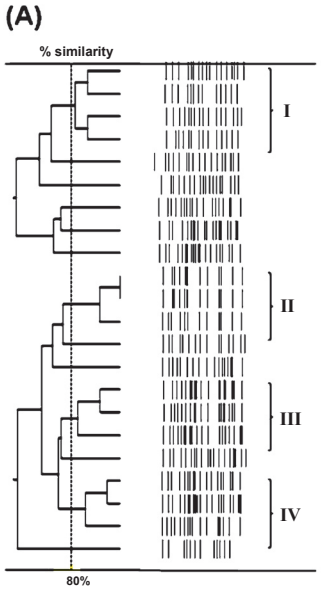
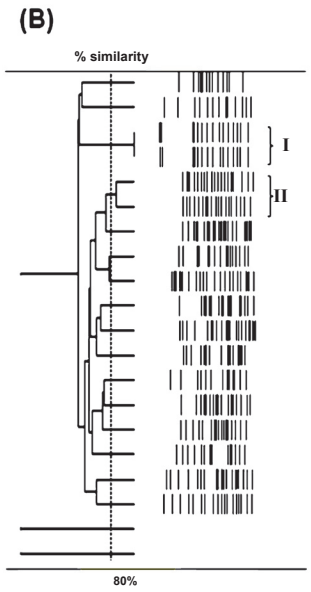


Fig. 1. Banding patterns of 22 *E. coli* isolates identified as ST131 using Achtman's MLST scheme **(A)** and 20 *E. coli* isolates identified as non-ST131 according to Achtman's MLST scheme, **(B)** obtained by rep-PCR. The dotted line in Fig. **1B** indicates 95 per cent similarity.



Isolate	Whittam's scheme ST
E08230	34
E09108	873
BDE0502	856
E08420	856
E09069	34
E06368	876
A05	34
E08344	34
E07294	34
H05	34
H08	34
H04	34
H24	34
H09	34
BDE0516	34
HYE0515	34
E09071	872
WKE0506	34
B060236	34
E07348	34
E08032	856
H30	H30



Isolate	ST	
	Achtman's scheme	Whittam's scheme
E05051	410	88
E08331	457	867
E07409	354	857
E08398	2037	857
B081373	38	303
WKE0506	131	34
H20	95	29
B090371	10	171
E08248	773	85
E07483	617	171
E08414	95	29
H21	744	171
B061647	1011	69
E08013	44	171
E09060	648	871
E08342	46	738
E08132	393	39
SCE0503	405	288
E08284	112	875
E08437	964	288

Fig. 2. 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 PFGE. The dotted lines in Figures indicate 80 per cent similarity.

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 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 enterotoxigeneic *E. coli* isolates identified as ST34 by Whittam's MLST scheme in a study by Steinsland *et al*[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*[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.

Acknowledgment

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (No. A085136).

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