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Evaluation of Ribotyping as Epidemiologic Tool for Typing *Escherichia coli* Serogroup O157 Isolates

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A total of 121 representative *Escherichia coli* O157:H7 and O157:NM (nonmotile) isolates were characterized by ribotype, phage type, verotoxin genotype, and genomic fingerprints generated by pulsed-field gel electrophoresis. Ribotyping was not able to discriminate between O157:H7 isolates, and phage typing and pulsed-field gel electrophoresis were the most valuable and discriminatory techniques.

Escherichia coli O157:H7 or O157:NM (nonmotile), an important food-borne pathogen (2) producing verotoxin (VT), is frequently associated with both hemorrhagic colitis and hemolytic-uremic syndrome (HUS) (5). Isolates of *E. coli* O157:H7 have few characteristics that can be used to distinguish one strain from the other. To assist in epidemiological investigations, a variety of phenotypic and genotypic typing schemes have been used, including bacteriophage typing (1, 6), VT genotyping (9, 15), plasmid profile analysis (8, 17), multilocus enzyme electrophoresis (18), and pulsed-field gel electrophoresis (PFGE) (2, 3). The present study was performed to evaluate ribotyping (13, 14) as a method for characterizing *E. coli* O157:H7 isolates and to compare it with the bacteriophage types, VT genotypes, and genomic fingerprints generated by PFGE after digestion with *Xba*I.

A total of 121 *E. coli* O157:H7 isolates were investigated and separated into three groups, as follows: group 1 included 85 human and nonhuman strains associated with outbreaks in Canada; group 2 included sporadic strains, 11 isolates associated with HUS, and 7 isolates not associated with HUS; and group 3 included 18 sporadic strains isolated from human and nonhuman sources from different countries.

Bacteriophage typing was performed by the methods and the scheme originally described by Ahmed et al. (1) and extended by Khakhria et al. (6).

VT genotypes were identified by the PCR-based methods described by Pollard et al. (9) and Tyler et al. (15).

Ribotyping was performed essentially as described previously by Tarkka et al. (14) by using the enzymes *Nco*I (16) and *Bam*HI, *Hin*dIII, and *Eco*RI (14) (Boehringer Mannheim, Laval, Quebec, Canada). The *E. coli* strains were grown overnight in brain heart infusion broth (Difco Laboratories, Detroit, Mich.), and the cells were harvested from 1.5-ml cultures. DNA was extracted by a standard sodium dodecyl sulfate lysis method; this was followed by phenol-chloroform extraction and ethanol precipitation (12). The *rrnB* rRNA operon of *E. coli* was used as a probe for ribotyping (4). An *E. coli* strain harboring the plasmid pKK3535 containing the *rrnB* insert was received from M. Altwegg, Department of Medical Microbiology, University of Zurich (7). The *rrnB* probe was labeled with digoxigenin-11-dUTP by using the DIG-DNA Labeling and Detection Kit (Boehringer Mannheim). As an internal marker, a supercoiled DNA ladder (Gibco BRL, Burlington, Ontario, Canada) linearized with *Pvu*II was multiplexed with each DNA sample. A second probe for detecting the marker was prepared by using an aliquot of the supercoiled DNA ladder. The same procedure for labeling the *rrnB* probe was used to label the ladder probe; however, digoxigenin-11-dUTP was substituted with fluorescein-12-dUTP (Boehringer Mannheim). Following electrophoresis, the DNA was transferred to Hybond N^+ (Amersham, Oakville, Ontario, Canada) by using a Posiblot Pressure Blotter (Stratagene, Professional Diagnostic Inc., Edmonton, Alberta, Canada), and the DNA was UV cross-linked to the membrane with a Stratalinker 2400 instrument (Stratagene).

Hybridization was performed at 65°C in the presence of both probes. Following high-stringency washes, the filters were again UV cross-linked. The *rrnB* probe was detected essentially as described in the instructions provided with the DIG-DNA Labeling and Detection Kit (Boehringer Mannheim). Detection was performed by chemiluminescence with Lumigen PPD (Boehringer Mannheim) as described by the manufacturer.

After autoradiographic detection of the *rrnB* probe, the alkaline phosphatase (AP) was inactivated in 50 mM EDTA (pH 8.0) at 85°C for 10 min. The fluorescein-labeled marker was then detected by substituting the anti-DIG-AP conjugate with the anti-fluorescein-AP conjugate (Boehringer Mannheim). Autoradiographs were developed on Hyperfilm MP (Amersham) and were analyzed by using the BioImage Workstation and Whole Band Analysis Software (Millipore, Ann Arbor, Mich.). Riboprobed and marker-probed autoradiographs were multiplexed in order to obtain the most accurate sizing of the bands for each sample.

PFGE was performed by using the LKB 2015 Pulsaphor system (LKB Pharmacia, Baie D'Urfe, Quebec, Canada). The strains were cultured in brain heart infusion broth (Difco Laboratories) for 18 h at 37°C. DNA for PFGE was prepared as described previously by Bohm and Karch (3) with the modifications of Barrett et al. (2). The agarose plugs were digested with *Xba*I (Boehringer Mannheim) as recommended by the manufacturer, and aliquots were subjected to electrophoresis for 26 h at 200 V, with pulse times of 5 to 50 s (linear ramping) and a temperature of 14°C.

A total of 121 strains of *E. coli* O157:H7 were ribotyped. Preliminary experiments with the restriction enzymes *Bam*HI, *Eco*RI, and *Hin*dIII with a variety of strains showed that *Hin*dIII gave potentially the most discriminating profile. However, each of the initial strains tested had identical ribotype

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TABLE 1. Sources and phenotypic and genotypic characteristics of the *E. coli* O157:H7 strains used in the study

Group	Strain	Source	Location[a]	Serotype	VT type (PCR)	Phage type	Ribotype (*Nco*I)	PFGE (*Xba*I)
1	87-2971	Human	ON	O157:H7	VT1, VT2	46	I	—[b]
1	89-2270	Human	MAN	O157:H7	VT1, VT2	14	I	P
1	88-2862	Human	ON	O157:H7	NEGATIVE	34	I	X
1	89-2352	Human	MAN	O157:H7	VT1, VT2	8	I	O
1	89-2407	Human	ALTA	O157:H7	VT1, VT2	2	I	B
1	89-2428	Human	NFLD	O157:H7	VT2	43	I	—
1	89-2503	Human	BC	O157:H7	VT1, VT2	1	I	O
1	89-2504	Human	BC	O157:H7	VT1, VT2	4	I	W
1	90-2184	Human	ALTA	O157:H7	VT1, VT2	26	I	R
1	90-2222	Human	PQ	O157:H7	VT2, VT2Va	39	I	S
1	91-2217	Human	NWT	O157:H7	VT1, VT2	32	I	F
1	91-2282	Human	ON	O157:H7	VT1	53	I	Y
1	91-2612	Nonhuman	PQ	O157:H7	VT1, VT2, VT2V[c]	40	I	V
1	92-3532	Human	ON	O157:H7	VT1, VT2Va	45	I	U
1	92-3543	Nonhuman	ON	O157:H7	VT1, VT2Va	23	I	N
1	93-2177	Nonhuman	BC	O157:H7	VT1, VT2	31	I	—
1	93-2437	Human	ON	O157:H7	VT1, VT2	33	I	Q
1	93-2669	Human	ALTA	O157:H7	VT1, VT2	10	I	T
2	84-1548	Human (HUS)	BC	O157:H7	VT1, VT2	4	I	A
2	84-1655	Human (HUS)	BC	O157:H7	VT1, VT2	1	I	B
2	84-1775	Human (HUS)	BC	O157:H7	VT1, VT2	1	I	C
2	85-0777	Human (HUS)	MAN	O157:H7	VT1, VT2	1	I	H
2	86-0719	Human (HUS)	ALTA	O157:H7	VT1, VT2	8	I	I
2	87-0855	Human (HUS)	NS	O157:H7	VT1, VT2	8	I	D
2	87-0865	Human (HUS)	ALTA	O157:H7	VT1, VT2	1	I	C
2	90-2090	Human (HUS)	ON	O157:H7	VT1, VT2	14	I	A
2	91-2434	Human (HUS)	MAN	O157:H7	VT1, VT2	32	I	F
2	91-2473	Human (HUS)	ALTA	O157:H7	VT1, VT2	14	I	J
2	91-2498	Human (HUS)	MAN	O157:H7	VT1, VT2	32	I	G
2	91-2535	Human	ON	O157:H7	VT1, VT2Va	67	I	N
2	92-3353	Human	PQ	O157:H7	VT2Va	70	I	L
2	92-3544	Human	ON	O157:H7	VT1, VT2Va	14	I	K
2	92-3545	Human	ON	O157:H7	VT1, VT2	1	I	M
2	93-2003	Human	ON	O157:H7	VT2	14	I	E
2	93-2014	Human	ON	O157:H7	VT2	14	I	C
2	93-2049	Human	PQ	O157:H7	VT1, VT2Va	23	I	K
3	91-2048	Unknown	Uruguay	O157:H7	VT1, VT2Va	39	I	—
3	91-2063	Unknown	England	O157:NM	VT1	65	I	—
3	92-3522	Nonhuman	United States	O157:H7	VT1, VT2	21	I	—
3	93-0782	Human	Canada	O157:H7	VT1, VT2Va	54	I	—
3	93-2003	Human	Canada	O157:H7	VT2	14	I	—
3	93-2140	Nonhuman	United States	O157:H7	VT1, VT2	14	I	—
3	93-2280	Human	United States	O157:H7	VT1, VT2	4	I	—
3	93-2317	Human	United States	O157:H7	NEGATIVE	39	I	—
3	93-2339	Human	United States	O157:H7	VT1, VT2	14	I	—
3	93-2372	Nonhuman	United States	O157:H7	VT2	2	I	—
3	93-2460	Human	Switzerland	O157:H7	VT1, VT2	14	I	—
3	93-2551	Human	Israel	O157:H7	VT2, VT2Va	2	I	—
3	93-2635	Human	Italy	O157:H7	VT1, VT2Va	8	I	—
3	93-2779	Human	Belgium	O157:H7	VT2Va	39	I	—
3	93-2782	Human	Belgium	O157:H7	VT2, VT2Va	4	I	—
3	94-2028	Human	Canada	O157:H7	VT1, VT2Va	8	I	—
3	94-2041	Nonhuman	Canada	O157:H7	VT2Va	4	I	—
3	94-2042	Nonhuman	Canada	O157:NM	VT2Va	31	I	—

[a] ON, Ontario; BC, British Columbia; MAN, Manitoba; ALTA, Alberta; NFLD, Newfoundland; PQ, Quebec; NWT, North West Territories; NS, Nova Scotia.
[b] —, not included in PFGE study.
[c] VT2V is an untypeable VT2 variant.

patterns specific to each of the three enzymes. By using *Hin*dIII, 85 strains were analyzed and were found to have identical ribotype patterns, each comprising eight fragments ranging in size from 0.3 to 17 kb. Even though *Hin*dIII was used successfully to ribotype non-O157 *E. coli* strains (14), it could not discriminate between strains within the O157:H7 serotype. *Nco*I had been reported to be a successful restriction enzyme in distinguishing between individual epidemic strains of *E. coli* O157:H7 (16). By using *Nco*I, 54 strains within the following three groups of *E. coli* O157:H7 were analyzed: group 1, 18 *E. coli* O157:H7 isolates belonging to different phage types from Canadian outbreak-associated human and

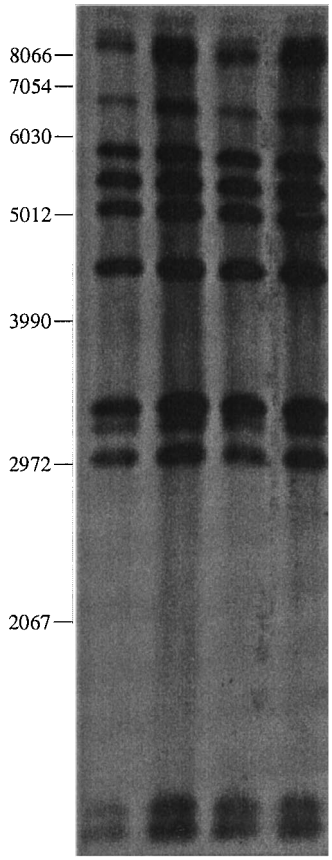

FIG. 1. Ribotype patterns of *E. coli* O157:H7 isolates obtained with *Nco*I. The results presented here represent those obtained for 54 strains in the study. Numbers on the left are in base pairs.

nonhuman isolates; group 2, 18 *E. coli* O157:H7 isolates including 11 isolates associated with HUS and 7 isolates not associated with HUS; and group 3, 18 *E. coli* O157:H7 isolates isolated from both human and nonhuman sources from a variety of countries. The results are summarized in Table 1. *Nco*I digestion produced 12 fragments ranging in size from 1.3 to 8.8 kb (Fig. 1). Even though these O157:H7 isolates were previously shown to be different VT genotypes and phage types and were isolated from different geographical locations, all 54 had identical ribotype patterns by using *Nco*I. In the present study, it was concluded that ribotyping was not able to discriminate between isolates within the O157:H7 serotype.

A total of 33 strains within groups 1 and 2 were investigated by comparing the numbers and sizes of fragments produced by PFGE. Twenty-five different PFGE patterns were observed, with fragments ranging in size from 50 to 500 kb (data not shown). Within group 1, PFGE was able to discriminate 14 of 15 different phage types. Within the second group, strains associated and not associated with HUS, PFGE discriminated 15 of the 18 strains. It was confirmed that PFGE was able to discriminate isolates within the O157:H7 serotype as reported by Barrett et al. (2).

In previous studies, ribotyping was shown to differentiate between different serogroups of *E. coli* and it appeared to be a suitable technique for typing *E. coli* isolates (14). In our investigations, although *E. coli* O55:H7 and O157:H7, strains that have a closely related clonal linkage (19), were clearly differentiated by ribotyping (data not shown), when it was limited to serogroup O157, ribotyping was not effective. In the present study, traditional and standard typing techniques such as phage typing proved to be more discriminatory than ribotyping. In addition, other Southern blot-based typing methods such as bacteriophage λ restriction fragment length polymorphism analysis (11) and restriction fragment length polymorphism analysis with Shiga-like toxin genes (10) have recently been proposed. Although molecular typing methods have continued to improve and are becoming accepted as the new standards, the use of just one technology alone (in this case, ribotyping) may not provide useful information. Any single method should not be used independently but is best combined with other molecular typing methods as well as those based on phenotypic characteristics. Although ribotyping is an acceptable typing method for many bacterial pathogens, it was not found to be useful for discriminating O157:H7 isolates. We report these findings with the intention that other laboratories will not use precious time and resources to evaluate a technique that does not provide an adequate level of discrimination. With the exception of some strains of phage types 43 and 31, PFGE did discriminate between isolates; however, it did not supply any more information than phage typing. In the present study, phage typing and PFGE appeared to be the preferred and most discriminatory typing techniques for *E. coli* O157:H7 strains. Since both ribotyping and PFGE are both costly and labor-intensive techniques, it is encouraging to see that phage typing, a relatively simple and inexpensive technique, is still effective and important.

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