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NOTES

Molecular Epidemiology of *Escherichia coli* O157:H7 by Pulsed-Field Gel Electrophoresis and Comparison with That by Bacteriophage Typing

UDO KRAUSE,[1] FIONA M. THOMSON-CARTER,[1,2]* AND T. HUGH PENNINGTON[1]

Department of Medical Microbiology[1] and Scottish National Reference Laboratory for Escherichia coli *O157,[2] Medical School, Foresterhill, Aberdeen AB9 2ZD, United Kingdom*

One hundred twenty-four *Escherichia coli* O157:H7 isolates were characterized by pulsed-field gel electrophoresis, bacteriophage typing, and PCR of verotoxin genes. Diversity indices obtained—0.786 for phage types and 0.987 for pulsed-field gel electrophoresis types—demonstrated that phage typing falls below the critical value (0.9) required for confident interpretation of results.

Verotoxin (VT) (Shiga-like toxin)-producing *Escherichia coli* O157:H7 is associated with outbreaks and sporadic cases of diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (5, 8, 9, 15, 16, 18, 20). Our knowledge of the epidemiology of *E. coli* O157:H7 infection is incomplete, and the source of the organism in sporadic cases has often been difficult to determine (16). Accordingly, many studies have been done to develop genotypic and phenotypic subtyping schemes (6, 15, 17). In 1987, a phage-typing scheme was developed by Ahmed et al. (1). In its initial form it used 16 bacteriophages and distinguished 14 types. Since then it has been further developed and used extensively for laboratory characterization of *E. coli* O157:H7. Currently 66 phage types are known; 24 types have been identified in Britain, although phage types 1, 2, 4, and 49 predominate (17).

Pulsed-field gel electrophoresis (PFGE) has been successfully used to characterize a range of bacterial species by detecting restriction fragment length polymorphisms (RFLPs) in their genomes (2, 10, 19). Böhm and Karch (6) analyzed 36 *E. coli* O157:H7 isolates by PFGE. Twenty-six strains were from Germany, and all had very similar RFLP profiles (4). In contrast, PFGE of more than 200 isolates from the United States showed that a group of strains associated with a large outbreak in western states in 1992 to 1993 could be distinguished from the remainder, which were diverse. Substantial differences were demonstrated between epidemiologically unrelated strains with identical phage types (4).

Scotland has a high incidence of infection with *E. coli* O157:H7 (16). However, the source and pattern of spread of strains remain obscure in many cases. Phage typing has been of limited value in these investigations because the majority of strains fall into a small number of types. Accordingly, we have investigated PFGE as a subtyping method.

A total of 124 *E. coli* O157:H7 strains isolated from humans and animals (16 bovine and 1 porcine) by diagnostic laboratories throughout Scotland from December 1992 to May 1994 received at the Scottish *E. coli* O157 Reference Laboratory, Department of Medical Microbiology, Aberdeen Royal Infirmary, were analyzed. Eighteen samples were known multiple isolates.

Isolates were identified as *E. coli* and characterized as *E. coli* O157:H7 by routine diagnostic methods. Phage types were determined by the standard method (1). All isolates were tested with primers specific for the genes encoding VT1 and VT2 as described previously (14).

PFGE profiles were obtained by the method of Thomson-Carter et al. (19). Digestion of genomic DNA was performed with *Xba*I (Promega, Southampton, United Kingdom) in accordance with the manufacturers' instructions: 30 U of *Xba*I at 37°C for 4 h. PFGE was performed in a CHEF DR-II apparatus (Bio-Rad) with 1% technical-grade agarose–Tris-borate buffer gels as described previously (19). Gels were electrophoresed for 22 h at 14°C at a constant voltage of 200 V with pulse times of 20 to 25 s with linear ramping. Lambda ladders (Bio-Rad) were used as markers.

PFGE profiles were compared with each other by using the Dice similarity coefficient and GelCompar Software (Applied Maths, Kortrijk, Belgium) (7). A phage type 49 strain was included in each gel for reference. Cluster analysis was done using the hierarchic unweighted pair group arithmetic average algorithm (maximum tolerance, 2.0%), and dendrograms were prepared. Known multiple isolates were not included in the dendrograms.

Twelve phage types were detected: 1, 2, 4, 8, 14, 28, 31, 32, 34, 40, 49, and 54. Animal isolates were phage types 54 (six bovine), 32 (one bovine), 14 (one bovine), 31 (one porcine), and RDNC (seven bovine). The distribution of strains among phage types was hyperbolic, with two types, 2 (43 isolates) and 49 (36 isolates), accounting for 63.7% of the study group. A significant percentage of the isolates (7.9%) did not fall into any phage type.

All isolates were probed for carriage of VT1 and VT2 genes (Fig. 1). The majority (89%) possessed the gene for VT2 only; the remainder (11%) possessed genes for both VT1 and VT2. In this group of isolates certain phage types were associated with certain VT profiles; e.g., isolates with phage type 49 were always VT1 negative and VT2 positive, whereas isolates with phage type 54 were VT1 positive and VT2 positive. No isolates which had the VT1 gene alone were identified.

Distinctive PFGE profiles were resolved for epidemiologically unrelated isolates. In contrast, identical profiles were

* Corresponding author. Mailing address: Scottish National Reference Laboratory for *E. coli* O157, Department of Medical Microbiology, Foresterhill, Aberdeen AB9 2ZD, United Kingdom. Phone: 40 224 663123, ext. 53819. Fax: 40 224 685604.

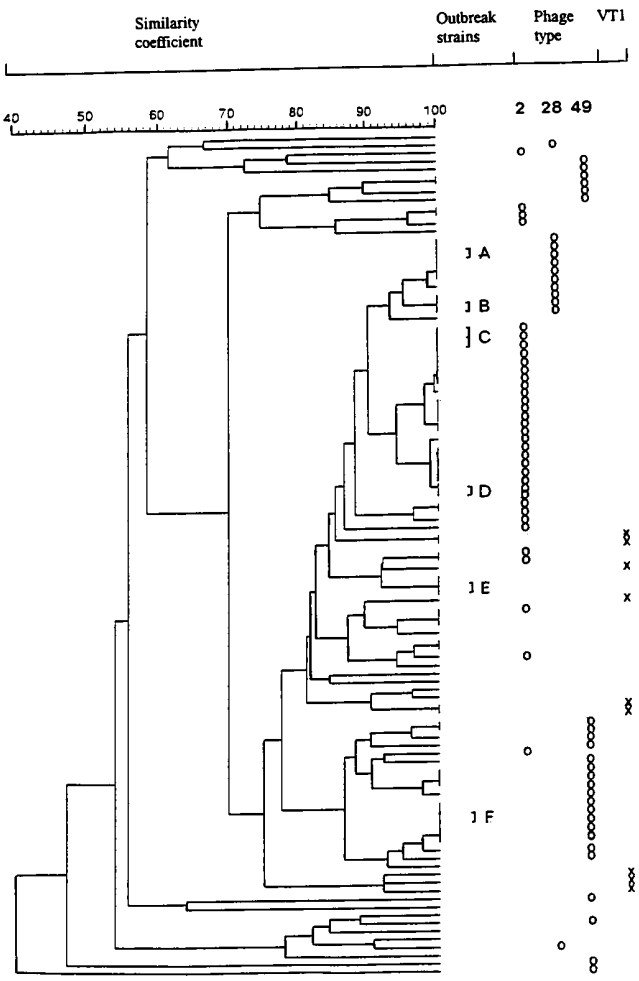

FIG. 1. Comparison of strain relationships based on cluster analysis of PFGE band patterns (dendrogram on the left) with phage type. The columns on the right indicate epidemiologically related strains (A, B, and C, multiple isolates from single families; D and F, common-source outbreaks; E, person or animal contact); strains with phage types 2, 49, and 28; and strains positive for the VT1 gene (all strains were VT2 positive).

obtained for epidemiologically related isolates (Fig. 1). (Samples with similarity coefficients of 95 to 100% were considered to be identical.) With a few exceptions samples with identical RFLP profiles belonged to the same phage type. However, no phage-type-specific profiles were apparent. Thus, the cluster analysis of RFLP patterns belonging to phage type 49 isolates yielded similarity coefficients in the range of 40 to 100%, and similarity coefficients for isolates with phage type 2 were in the range of 70 to 100%. Cluster analysis of all isolates used in this study was performed (Fig. 1). All the epidemiologically related isolates fell into clusters with 95 to 100% similarity. Usually only one phage type was present within these clusters. However, some isolates with identical RFLP patterns had different phage types; thus, three VT1-negative, VT2-positive strains with indistinguishable PFGE profiles were phage types 40, RDNC, and 49.

The development of DNA fingerprinting schemes for the typing of bacteria has in part been stimulated by the view that phage typing no longer occupies the "gold standard" position as a high-resolution discriminator between strains. Thus, for *Staphylococcus aureus* it has been described as "a system that lacks any biological basis. . .plagued by nontypable isolates and by unpredictable variability among typable ones" (13). PFGE of genomic DNA digested by rare cutter restriction enzymes has been widely used as a DNA fingerprinting method and has shown itself to have advantages over other DNA-based methods, such as plasmid analysis, which in essence tracks plasmids rather than their hosts; conventional RFLP analysis, which requires the analysis of complex banding patterns; and ribotyping, which is labor-intensive and slow (3, 19).

In this study we have compared phage typing and PFGE as methods for characterizing *E. coli* O157:H7 strains. All strains referred to the Scottish National Reference Laboratory between the end of December 1992 and the end of December 1993 were examined; because most Scottish human isolates were referred to the laboratory for detailed characterization, the collection can be deemed to contain material representative of all strain types causing human infections in that part of the United Kingdom during the period. Subclones were defined as being marked by PFGE band pattern Dice coefficient values of less than 95%. Comparison of the results with those of phage typing showed that PFGE was much more discriminatory. Thus, calculation of the diversity index, Simpson's *D*, (11) gave a value of 0.786 for phage types and 0.987 for PFGE types. Simpson's *D* is directly related to richness (the number of types in sample) and evenness (the relative distribution of individual strains among the different types) (12). It has been suggested by Hunter and Gaston (11) that a value of greater than 0.9 "would seem to be desirable if the typing results are to be interpreted with confidence." The hyperbolic distribution of phage types accounts for the low score of Simpson's *D*, which falls well below this critical value.

The quantification of diversity is also dependent on two value judgments: whether the selected classes are different enough to be considered separate types and whether the objects in a particular class are similar enough to be considered the same type (12). For the first of these a particularly relevant consideration is that both phage and PFGE type definitions are to a degree arbitrary and based on rule of thumb. This is because important information about the biological basis of the differences detected by these methods is lacking. An important example of this deficiency is our inability to say whether differences in PFGE profile are due to sequence rearrangements, the addition or deletion of DNA, or base substitution within cleavage sites. Further work is required to diminish the empirical elements of both typing methods.

The demonstration that strains belonging to a single phage type had PFGE similarity coefficients as low as 40% and that a group of strains had identical PFGE profiles but different phage types clearly indicates that a major dissociation can exist between phage type and genotype and that their evolution has proceeded along different pathways. In the absence of information on the stability of phage type over time in strains growing in their natural environment, the practical significance of the results is unclear. They suggest, at least, that caution be used when using phage type to index strain relationships.

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REFERENCES

1. **Ahmed, R., C. Bopp, A. Borczyk, and S. Kasatiya.** 1987. Phage-typing scheme for *Escherichia coli* O157:H7. Infect. Dis. **155:**806–809.
2. **Arbeit, R. D., M. Arthur, R. Dunn, C. Kim, R. K. Selander, and R. Goldstein.** 1990. Resolution of recent evolutionary divergence among *Escherichia coli* from related lineages: the application of pulsed field electrophoresis to molecular epidemiology. J. Infect. Diseases **161:**230–235.
3. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995.

Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551–555.
4. **Barrett, T. J., H. Lior, J. H. Green, R. Khakhria, J. G. Wells, B. P. Bell, K. D. Green, J. Lewis, and P. M. Griffin.** 1994. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. J. Clin. Microbiol. **32:**3013–3017.
5. **Beutin, L., D. Geier, H. Steinrueck, S. Zimmermann, and F. Scheutz.** 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. **31:**2483–2488.
6. **Böhm, H., and H. Karch.** 1992. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J. Clin. Microbiol. **30:**2169–2172.
7. **Dice, L. R.** 1945. Measures of the amount of ecologic association between species. Ecology **26:**297–302.
8. **Donnenberg, M. S., and J. B. Kaper.** 1992. Enteropathogenic *Escherichia coli*. Infect. Immun. **60:**3953–3961.
9. **Gunzer, F., H. Böhm, H. Rüssmann, M. Bitzan, S. Aleksic, and H. Karch.** 1992. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J. Clin. Microbiol. **30:**1807–1810.
10. **Harsono, K. D., C. W. Kaspar, and J. B. Luchansky.** 1993. Comparison and genomic sizing of *Escherichia coli* O157:H7 isolates by pulsed-field gel electrophoresis. Appl. Environ. Microbiol. **59:**3141–3144.
11. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
12. **Huston, M. A.** 1994. Biological diversity. Cambridge University Press, Cambridge.
13. **Kreiswirth, B., J. Kornblum, R. D. Arbeit, W. Eisner, J. N. Maslow, A. McGeer, D. E. Low, and R. P. Novick.** 1993. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. Science **259:**227–230.
14. **Pollard, D. K., W. M. Johnson, H. Lior, S. D. Tyler, and K. R. Rozee.** 1990. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J. Clin. Microbiol. **28:**540–545.
15. **Samadpour, M., L. M. Grimm, B. Desai, D. Alfi, J. E. Ongerth, and P. I. Tarr.** 1993. Molecular epidemiology of *Escherichia coli* O157:H7 strains by bacteriophage λ restriction fragment length polymorphism analysis: application to a multistate foodborne outbreak and a day-care center cluster. J. Clin. Microbiol. **31:**3179–3183.
16. **Sharp, J. C. M., J. E. Coia, J. Curnow, and W. J. Reilly.** 1993. *Escherichia coli* O157 infections in Scotland. J. Med. Microbiol. **40:**3–9.
17. **Smith, H. R., and S. M. Scotland.** 1993. Isolation and identification methods for *Escherichia coli* O157 and other verocytotoxin producing strains. J. Clin. Pathol. **46:**10–17.
18. **Thomas, A., H. Chart, T. Cheasty, H. R. Smith, J. A. Frost, and B. Rowe.** 1993. Verocytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in the United Kingdom: 1989–91. Epidemiol. Infect. **110:**591–600.
19. **Thomson-Carter, F. M., P. E. Carter, and T. H. Pennington.** 1993. Pulsed-field gel electrophoresis for the analysis of bacterial populations, p. 251–264. *In* R. G. Kroll, A. Gilmour, and M. Sussman (ed.), New techniques in food and beverage microbiology. Blackwell Scientific Publications, Oxford.
20. **Whittam, T. S., M. L. Wolfe, I. K. Wachsmuth, F. Ørskov, I. Ørskov, and R. A. Wilson.** 1993. Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. Infect. Immun. **61:**1619–1629.