PFGE TYPING OF HUMAN CASE AND FOOD ISOLATES OF *E. COLI* O157:H7 IN NEW ZEALAND

FINAL REPORT

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by

Angela Cornelius
Brent Gilpin
Carolyn Nicol

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Institute of Environmental Science & Research Limited
Christchurch Science Centre
Location address: 27 Creyke Road, Ilam, Christchurch
Postal address: P O Box 29 181, Christchurch, New Zealand
Website: www.esr.cri.nz

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Stephen On
Food Safety Programme Leader

Angela Cornelius
Project Leader

Ellen Podivinsky
Peer Reviewer

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SUMMARY

From March to September 2006, 25 isolates were uploaded to the PulseNet USA *E. coli* O157:H7 pulsed field gel electrophoresis (PFGE) database with the *Xba*I:*Bln*I pattern EXHX01.0074:EXHA26.0569. Although this pattern is relatively common in the US database, this number of isolates suggests a potential common source outbreak. USDA-FSIS found *E. coli* O157:H7 isolates from two meat-processing plants with two similar *Xba*I:*Bln*I patterns (EXHX01.0074:EXHA26.0569 and EXHX01.1401:EXHA26.0569). One common link between these meat-processing plants is that both sourced some of their meat from New Zealand.

As a consequence of the isolations in the US, in April 2006 the NZFSA and, independently, ESR (PulseNet Aotearoa) received an urgent request from the USDA-FSIS and US-CDC, requesting information on the prevalence of this pattern amongst New Zealand *E. coli* O157:H7 isolates.

As the New Zealand database contained only limited data, responding to this request required the analysis by PFGE of over 200 additional isolates. Comparisons were made with the *Xba*I profiles of 203 human isolates and 229 meat isolates.

Of these, 12 human isolates and three meat isolates had *Xba*I patterns that were indistinguishable from the US pattern EXHX01.0074. *Bln*I profiles were generated for these isolates and all differed from the USA pattern EXHA26.0569. No isolates were identified with the XbaI pattern EXHX01.1401.

Consistent with US database, the *Xba*I pattern EXHX01.0074 appears relatively common in New Zealand isolates (5.9%). Furthermore it appears relatively stable having been isolated over at least five years, with no appreciable genetic changes.

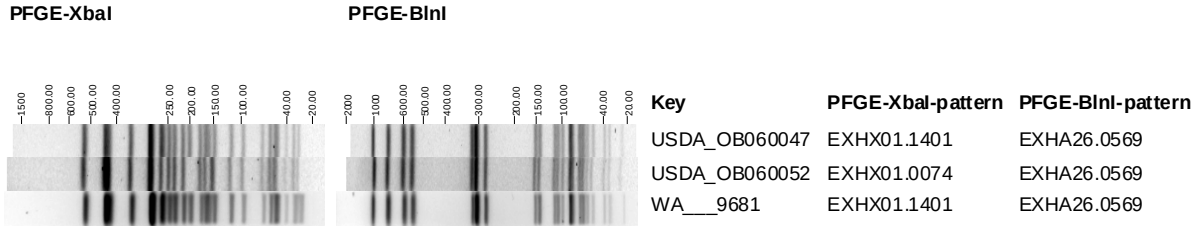
A further 10 human and 34 meat isolates, mostly with similar *Xba*I profiles, were genotyped using *Bln*I. Two of the meat isolates had *Bln*I patterns that were indistinguishable from the USA pattern EXHA26.0569. Their *Xba*I profiles however differed from the USA pattern.

All of the New Zealand isolates were distinguishable from the USA patterns EXHX01.0074:EXHA26.0569, and EXHX01.1401:EXHA26.0569. There is no evidence to indicate that the *E. coli* O157:H7 isolates recovered from the US meat-processing plant came from New Zealand meat.

1 BACKGROUND

In April 2006 the New Zealand Food Safety Authority (NZFSA) and, independently, ESR (PulseNet Aotearoa) received an urgent request from the USDA-FSIS and US-CDC, via PulseNet USA, for information on the occurrence in New Zealand of two pulsed field gel electrophoresis (PFGE) patterns for *E. coli* O157. These patterns, illustrated in Figure 1, were from two isolates from ground beef containing both US and New Zealand beef, and the patterns have also been implicated in a cluster of human illnesses in the United States of America. The patterns are relatively common in the US and one partial pattern (using one enzyme rather than two) is also common in New Zealand. However, the databases in New Zealand are not well populated with data, and as a consequence NZFSA was unable to provide a robust answer to the US request.

Figure 1: PFGE patterns of US *E. coli* O157:H7 isolates



It was therefore considered imperative that NZ human and food (meat) isolates be PFGE-typed with urgency, using two appropriate enzymes, to answer the current request, and to facilitate a more rapid response to further requests.

1.1 Current Best Practice for Interpreting PFGE Images

The objective of this study was to establish the prevalence in New Zealand of two *E. coli* O157:H7 PFGE patterns so that this information could be reported to PulseNet USA. An important step in this process was to ensure that PFGE results were interpreted in New Zealand, as they would have been by PulseNet USA. A brief review of the recent literature was undertaken to establish current best practice and identify what criteria PulseNet USA currently use to report PFGE patterns.

In 1995 Tenover *et al.* published a paper suggesting guidelines for the interpretation of PFGE data. In the absence of alternative papers, the "Tenover criteria" have for the last decade been the *defacto* standard worldwide. The "Tenover criteria" suggested that profiles differing from each other by up to three bands should be considered closely related, and up to six bands possibly related. The basis of this was that a point mutation in a restriction site could result in loss of that site with two bands in one isolate merging to form a larger band – i.e. a "three band difference". Using this logic, a three band difference could be the result of a single genetic event, and therefore isolates could be closely related.

These guidelines were developed on the basis of comparison of nosocomial pathogens, particularly in situations of ongoing transmission. These guidelines also assume that all fragments are visible in a gel, that the plasmid content is stable, and that most mutations are

point mutations. For foodborne transmission of pathogens, and for *E. coli* O157 in particular, all three of these assumptions are flawed. Addressing these in order:

- PFGE usually only resolves a limited number of bands. Theoretical digestion of sequenced genomes of *E. coli* O157 strains should generate 41 fragments with *Xba*I and 31-33 fragments with *Bln*I. Some smaller bands run off the gel, while others are too similar in size and comigrate. As a consequence only half to three quarters of these bands can usually be distinguished by PFGE analysis (see Appendix 1).
- Plasmids are common in foodborne pathogens. If digested by a restriction enzyme they will migrate in a PFGE gel as a function of their size, just like chromosomal fragments. Often if they are multiple copy plasmids they are observed as intense bands. However if they are not in a linear conformation, their migration is unpredictable. Large undigested plasmids may not leave the wells of a gel, but may also be visible almost anywhere in the gel. Large megaplasmids are common in foodborne pathogens.
- Kudva *et al.* (2002) demonstrated that PFGE diversity in *E. coli* O157 is primarily attributed to insertions and deletions, not to point mutations as the "Tenover criteria" assume.

Under the "Tenover criteria", single band differences should not be possible. They are however commonly observed, either because of the other bands involved not being resolved, or the presence of plasmids.

Barrett *et al*. (2006) have recently re-evaluated the "Tenover criteria" in light of practical experiences of PulseNet USA. Their revised recommendations are:

- When any differences in PFGE patterns are observable, the patterns should be reported as different.
- Patterns that are indistinguishable should be reported as such, not as identical.

Interpretation of patterns does however require adherence to the following steps:

1. The gel must be of sufficient quality to be properly interpreted.
2. The diversity of the organism under consideration must be considered. Even in an organism with high diversity, some clonal populations may exist.
3. The outbreak setting should be considered. More variability is likely in ongoing transmission than suspected point source.
4. The most important factor is how the laboratory data fit with the epidemiologic and environmental information. PFGE results alone cannot establish an epidemiological connection between isolates.

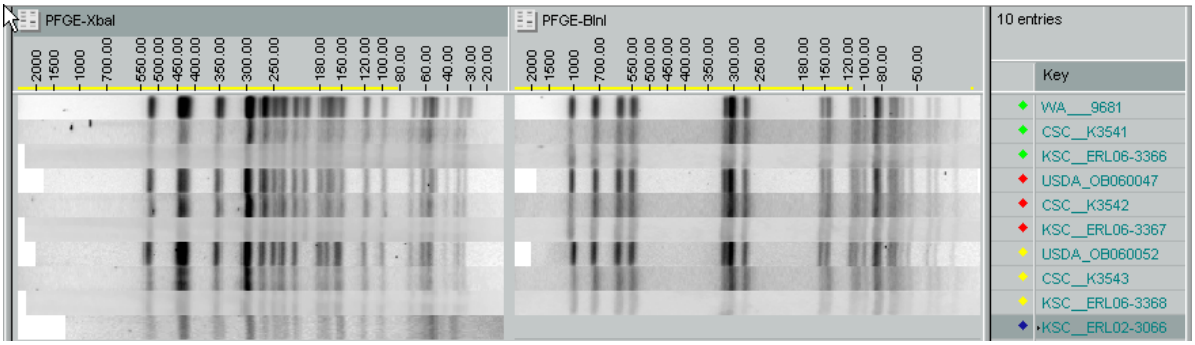
Current best practice at PulseNet USA requires indistinguishable patterns using two enzymes for isolates to be considered indistinguishable by PFGE. The application of a second enzyme is well supported in the literature, pioneered perhaps by On *et al*. (1998). The validity of the second enzyme is of course subject to epidemiological investigation, and dependent on the nature of the outbreak. For common patterns a second enzyme is even more important.

2 RESULTS

2.1 Analysis of US Isolates

As part of this project cultures of three isolates with the PFGE patterns of interest were sent from the US so that inter-laboratory variations were overcome. Figure 2 illustrates the patterns of the three isolates analysed in three locations (US, Christchurch Science Centre and Kenepuru Science Centre). Entries with the same colour marker are replicates of the same isolate. Entries with the prefixes CSC and KSC were analysed at Christchurch and Kenepuru Science Centres respectively. Replicates run in the US have either WA or USDA prefixes. The entry with a blue marker is from a human isolate in 2002 showing the EXHX01.0074 pattern is stable over a number of years.

Figure 2: Patterns of US isolates analysed in three laboratories



All of the replicates from each isolate produced the same pattern in all three laboratories.

Recommendation: Based on this analysis and provided PFGE has been performed to acceptable standard, images of patterns for which information has been requested is sufficient for comparison. Therefore cultures of representative isolates are not required. This is accepted practice in the US.

This recommendation is supported by the long delay in shipping cultures internationally. In this case the request was made in May and the cultures did not arrive until September. The time involved in applying for, and obtaining, an export permit for the isolates was a major cause of the delay. Obtaining these permits for *E. coli* O157 is likely to be very difficult, if not impossible, in the future as this group of organisms has been labelled an agent of bioterrorism.

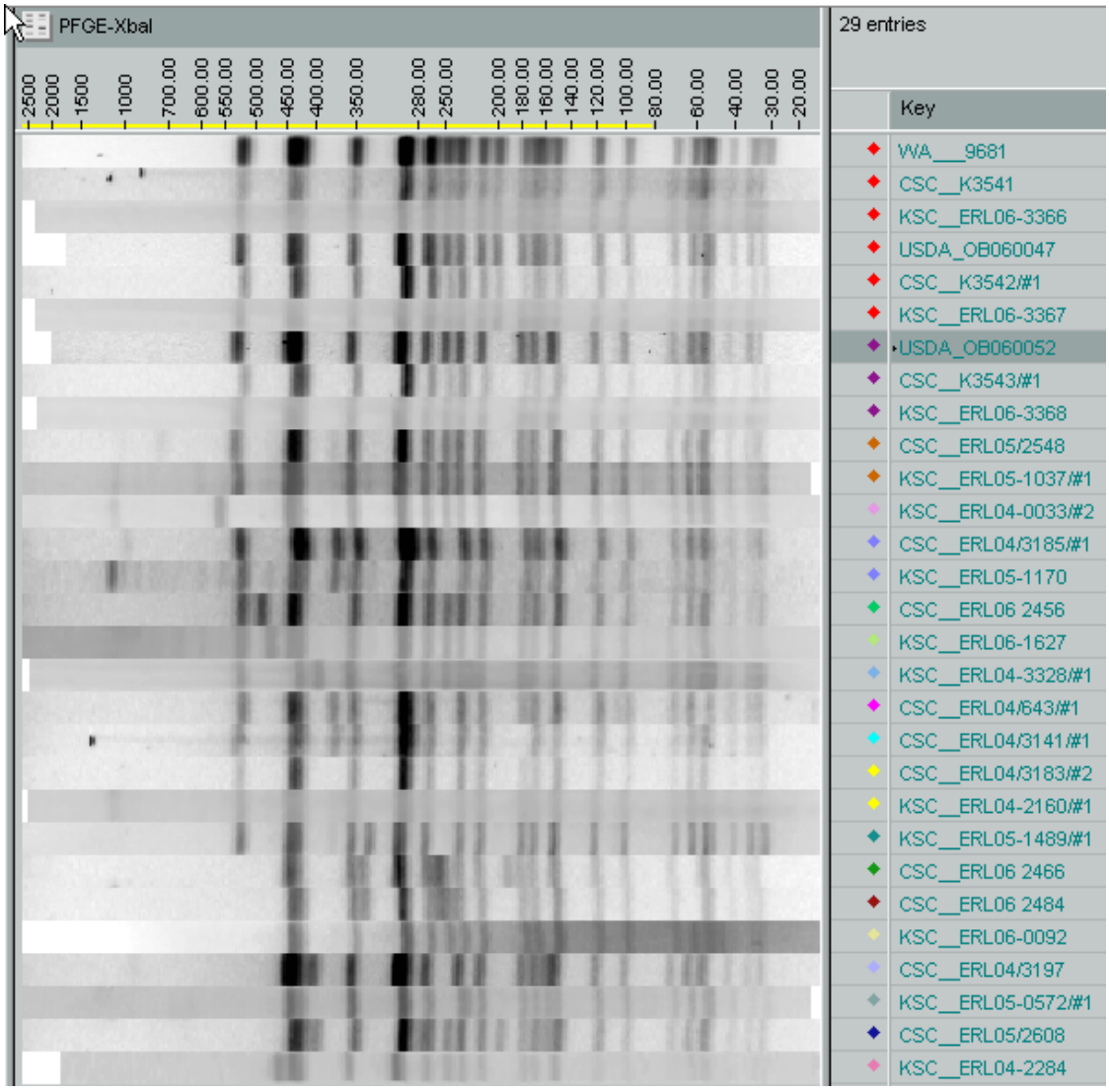
2.2 *Xba*I Results (Stage 2)

A list of isolates was agreed between the NZFSA and ESR and contained 205 human and 237 meat isolates (including 95 isolates from AgResearch) received at ESR Enteric Reference Laboratory between 2004 and 2006 and for which sufficient information is available to adequately identify the source of the isolate. Of these, two meat isolates were identified as non-toxigenic and another five meat and two human isolates had become non-viable. The remaining 229 meat and 203 human isolates were digested using *Xba*I and the bands separated by PFGE using the PulseNet protocol:
http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf

Isolates with patterns that appeared similar to, or indistinguishable from, the US isolates were analysed alongside the US isolates.

All isolates had patterns that were distinguishable from the US pattern EXHX01.1401. Three meat and 12 human isolates had patterns indistinguishable from the US pattern EXHX01.0074. A further 34 meat and 10 human isolates had *Xba*I patterns similar to the US patterns. Figure 3 illustrates the *Xba*I patterns of a selection of isolates representing the US isolates (analysed in US, at CSC and at KSC) and New Zealand isolates with indistinguishable and similar patterns from both meat and human sources. Isolates with prefixes CSC and KSC are New Zealand meat and human isolates respectively. US isolates have the prefixes WA or USDA. The US isolates with pattern numbers EXHX01.1401 and EXHX01.0074 have red and purple markers respectively. The two NZ isolates shown below the US isolates have patterns indistinguishable from EXHX01.0074. The remaining isolates represent similar patterns. Isolates with indistinguishable patterns have been assigned the same colour marker. Meat and human isolates of each pattern are included where possible.

Figure 3: *Xba*I patterns for *E. coli* O157:H7 isolates



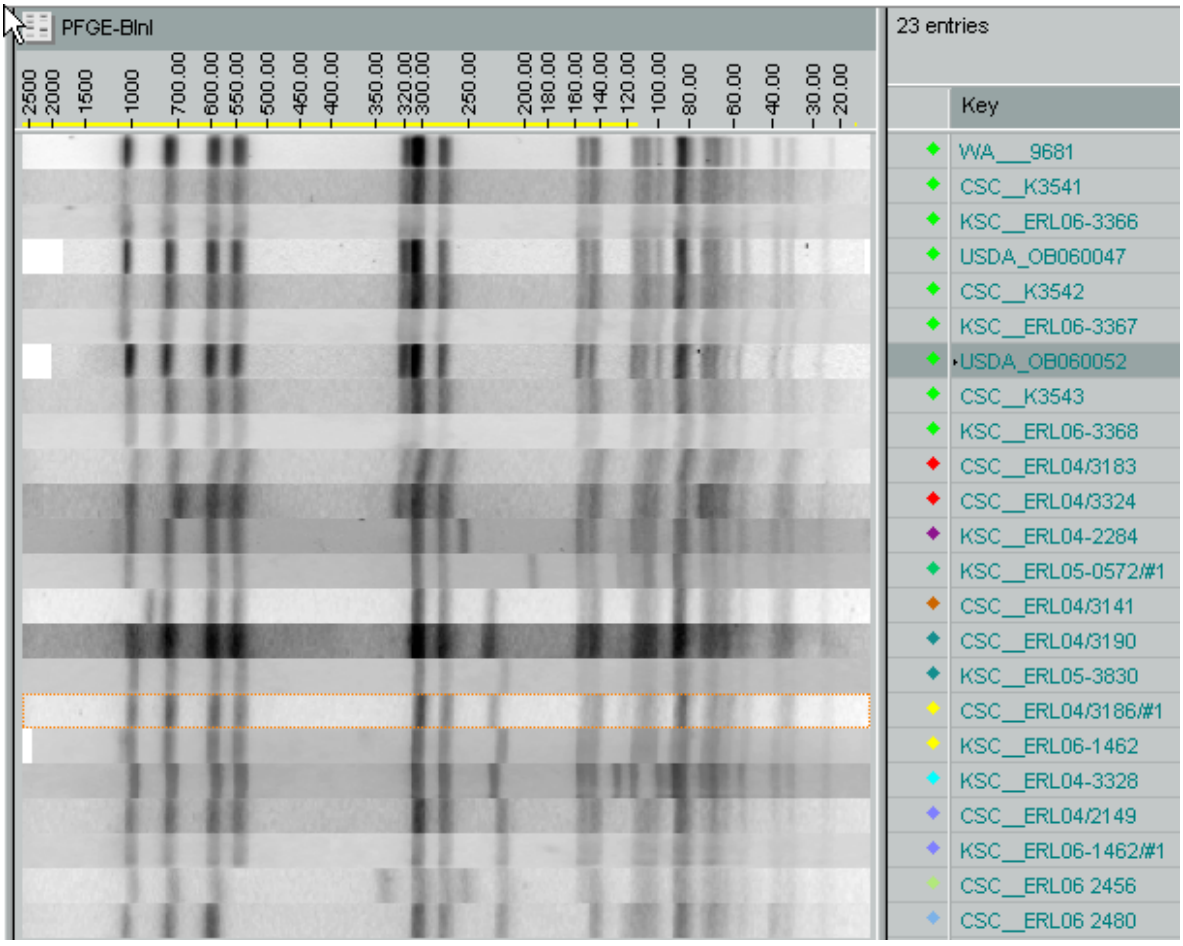
2.3 *Bln*I Results (Stage 3)

All of the 37 meat and 22 human isolates that had *Xba*I patterns that were indistinguishable from, or similar to, the US patterns were digested using *Bln*I and separated by PFGE using the PulseNet Protocol:
http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf.

The US isolates were analysed in each batch of isolates.

Two meat isolates had patterns that were indistinguishable from the US pattern EXHA26.0569. Figure 4 illustrates the *Bln*I patterns of a selection of isolates representing the US isolates (analysed in US, at CSC and at KSC) and NZ isolates from both meat and human sources. The US isolates have fluorescent green markers and the two NZ meat isolates with patterns indistinguishable from these have red markers. Isolates with prefixes CSC and KSC are NZ meat and human isolates respectively, and US isolates have the prefixes WA or USDA. Isolates with indistinguishable patterns have been assigned the same colour marker and meat and human isolates of each pattern are included where possible.

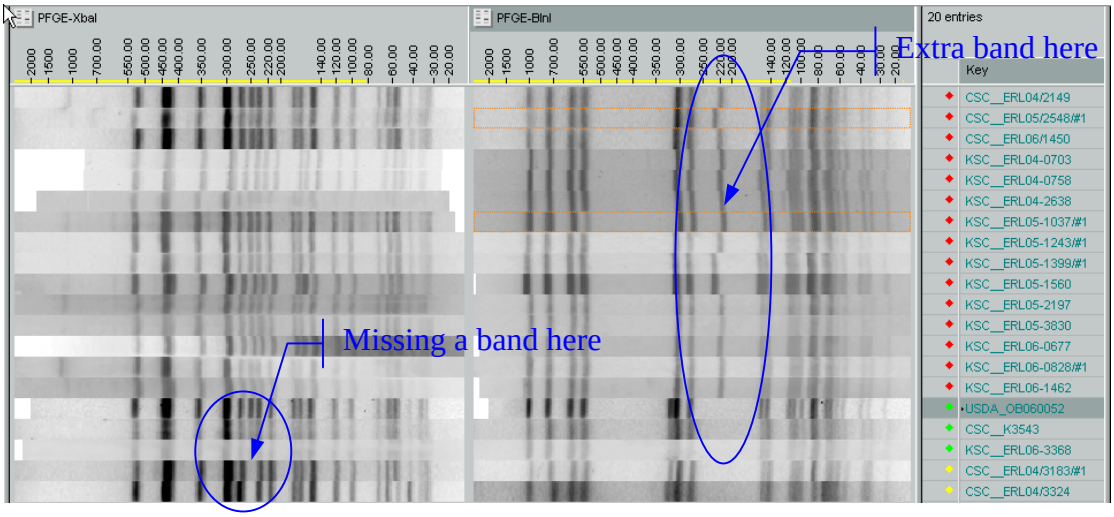
Figure 4: *Bln*I patterns for *E. coli* O157:H7 isolates



2.4 Combined *Xba*I:*Bln*I Patterns

All isolates had combined *Xba*I:*Bln*I patterns that were distinguishable from the US isolates. Figure 5 illustrates the *Xba*I and *Bln*I patterns for isolates that had *Xba*I or *Bln*I patterns that were indistinguishable from the US isolates. NZ isolates with *Xba*I patterns that were indistinguishable from US pattern EXHX01.0074 have a red marker. The US isolates analysed in the US, at CSC and at KSC have a fluorescent green marker. New Zealand isolates were *Bln*I patterns that were indistinguishable from US pattern EXHA26.0569 have a yellow marker.

Figure 5: *Xba*I:*Bln*I patterns for isolates with *Xba*I or *Bln*I patterns indistinguishable for the US Isolates



3 CONCLUSIONS

All of the New Zealand isolates were distinguishable from the USA patterns EXHX01.0074:EXHA26.0569, and EXHX01.1401:EXHA26.0569. There is no evidence to indicate that the *E. coli* O157:H7 isolates recovered from the US meat-processing plant came from New Zealand meat.

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APPENDIX 1: PFGE THEORETICAL DIGESTS

Escherichia coli O157:H7 EDL933 - 41 fragments with *Xba*I, 33 with *Bln*I
Escherichia coli O157:H7 VT2-Sakai, 41 fragments with *Xba*I, 31 with *Bln*I

The schematic gels below illustrate that less fragments are likely to be visualised. Fragments below 20,000 bp will usually run off a gel.