Genetic Typing of Campylobacter spp. Isolated From Retail Packs of Poultry in Northern Ireland.

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BACKGROUND. Reports of *Campylobacter* infections in Northern Ireland have exceeded those of salmonellosis since 1991 and *Campylobacter* spp. are now the major cause of food-borne infections in the province (Anon. 1995). However outbreaks are rare and cases mainly sporadic in nature. Determining the cause of sporadic outbreaks is problematical due to the relatively fragile nature of the organism and the difficulty in comparing isolates when obtained. This contrasts with the serotyping system established for *Salmonella*, for example, which allows sporadic occurrences of specific serotypes to be monitored (Doyle and Cliver 1990). It has been reported that meats, especially poultry, are often implicated in cases of campylobacter food-poisoning (Doyle 1990) hence a survey of local retail packs of poultry was undertaken (Madden *et al*, in press). This survey revealed comparatively low levels of campylobacters in retail packs (38%) but all of the packs were from one local producer. Isolates from the packs were all successfully typed using genetic methods. To further assess the uses of genetic typing a larger survey was designed and conducted.

OBJECTIVES. Retail packs of chicken (128), from as wide a geographical range of producers as possible, were purchased in the Belfast area analysed for campylobacters. Isolates from the packs were typed using the random amplified polymorphic DNA (RAPD) method (Welsh and McClelland 1990, Williams *et al.* 1990), followed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on the method of Nachamkin *et al.* (1993). The aim was to isolate campylobacters and hence determine the level of contamination of the retail packs, and then devise a rapid scheme for obtaining a genetic sub-type.

METHODS. Samples (6) were always purchased on a Tuesday and transferred to the laboratory for analysis within 1h. Sample size was sometimes less due to a limited range of products being on sale. A total of 128 samples was analysed (June 1996-April 1997). In the lab 10g of material from each pack, in duplicate, was added to 90 ml Preston broth with FBP and incubated (microaerophilic, 42^0C, 24h). Each broth was then streaked onto Preston agar and incubated as above. Typical colonies were picked off and biotyped to speciate the isolates. One isolate from each positive enrichment was stored for subsequent sub-typing analyses. DNA isolation was based on the preparation of cell lysates. A loopful of culture was boiled for 10 min and the cell debris removed by centrifugation. Lysates were prepared as soon as cultures were isolated then stored frozen at -20^0C until the survey was completed. The DNA concentration was estimated spectrophotometrically (260nm) prior to PCR amplification. RAPD typing was performed as previously described (Madden *et al.* 1996). PCR-RFLP typing was by the method of Nachamkin *et al.* (1993), with a primer set amplifying the *flaA* gene and the subsequent 1.7 kilobase amplicon being digested with *Dde*1 restriction endonuclease. Subsequently DNA extractions used the CTAB method of Wilson (1987) or Nucleon extraction kits (Amersham Pharmacia, Little Chalfont, GB).

RESULTS AND DISCUSSIONS. Overall 128 packs were sampled, representing 5 distinct areas within the European Community, and 91 packs contained *Campylobacter* spp.. This level (71.1%) is significantly higher than was found in our previous study of a single processor (Madden et al, 1998) where 38% of packs contained campylobacters and greater than that observed by Flynn *et al.* (1994) who found 64.7% of packs of chicken wings (n=153) purchased in Northern Ireland were positive. Comparison with the former survey is not appropriate due to its narrow scope . However the differences noted with the latter survey could be due to methodological differences, including the part of the carcass sampled, or seasonal variations. It could also be due to the age of packs purchased as our sampling was arranged to ensure packs were sampled as soon as possible after arrival at the supermarket, following preliminary trials in our laboratory which showed significant loss of viability of *Campylobacter jejuni* occurred during simulated chilled display. Analysis of stored lysates prepared from the 182 isolates obtained commenced with RAPD and all of the organisms obtained were typable by this method. Using the patterns obtained isolates from duplicate enrichment broths were compared . If identical types were observed (Fig. 1) then only one isolate was subjected to further analysis in order to reduce the workload. RAPD was found to be highly discriminating (Madden *et al*, 1998) hence it was considered unlikely that further study would resolve identical RAPD types into separate sub-types. Initial trials suggested that packs would mainly contain one dominant sub-type however when work was completed 45 packs yielded the same isolate form each enrichment whereas a different RAPD type was isolated from 46. Overall, therefore 137 isolates were examined by PCR-RFLP of the *flaA* gene.

Stored lysates were again used and 93 isolates successfully typed, Fig. 2. The remaining 44 isolates were untypable and further study showed that these isolates were not evenly distributed throughout the producers sampled but appeared to have distinct geographical origins. Thus whilst 19% of local (Northern Ireland) campylobacters (n=100) were untypable the rate varied from 7% of Eire isolates (n=20) to 50% in Scotland (n=24) and England (n=30). Only 8 campylobacters were obtained from French poultry but all proved to be untypable. Therefore the *Campylobacter* isolates in certain geographically distinct areas appear to possess physiological properties which mean that the use of stored lysates for PCR-RFLP will not be successful.

Attention was then turned to determine which DNA extraction methods would allow PCR-RFLP typing to be applied to all isolates. Fresh cultures were prepared and lysates obtained which were subjected to PCR with Nachamkin's *flaA* primers on the same day. Twenty two (50%) of the 44 untypables yielded product and could hence be typed. Thus lysates from these organisms are not stable under the storage conditions used but successful typing could be conducted with fresh lysates. DNA was obtained from 18 (34%) of the 44 untypables by CTAB extraction but the DNA was seen to lack stability as after 4 months good quality DNA, as visualised on 1% agarose gels was no longer visible from some isolates. Using Nucleon kits 23 untypables were investigated and all produced good quality DNA which remained intact after 2 months of storage at -20^0C, (Fig. 3).

Overall good quality DNA was extracted from 38 of the untypables using CTAB or Nucleon kits. Subsequently 35 untypables yielded product with universal 16S rRNA primers and 30 of these also yielded product with Nachamkin's *flaA* primers. Thus of the original

137 isolates 123 were *flaA* typed and 5 were untypable using Nachamkin's primers. Three yielded DNA but no product was obtained with the *flaA* or 16S primers, and the remaining 6 campylobacters are being investigated to define appropriate DNA extraction protocols. Untypable isolates were all DNAse negative hence this was not the cause of untypability.

CONCLUSIONS. RAPD from stored cell lysates was the most rapid and simple genetic typing scheme applicable to *Campylobacter* spp. isolated from retail packs of chicken. PCR-RFLP typed 90% of isolates with 4% being untypable, however in addition to the stored lysates this method required a combination of fresh isolates and Nucleon kit DNA extractions to achieve this. The remaining 6% comprised campylobacters which did not yield DNA suitable for the assay. Untypability seemed to be geographically related, suggesting distinct populations of campylobacters may exist in certain regions. This will be investigated in the next phase of the research when a relatedness survey of the gel patterns obtained will be conducted.

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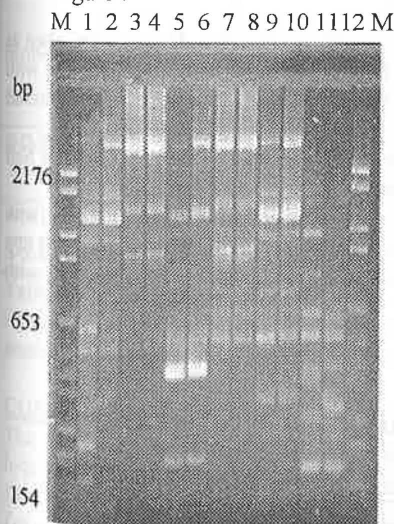
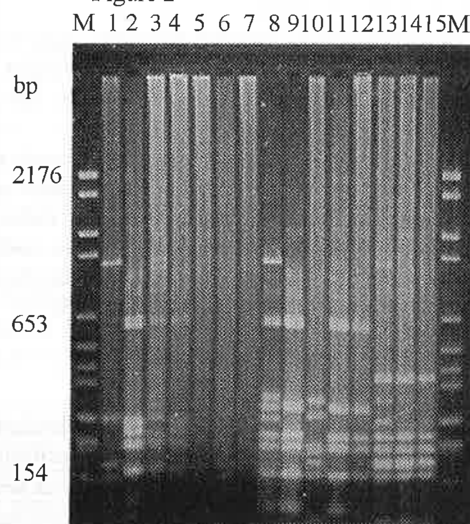
Figure 1



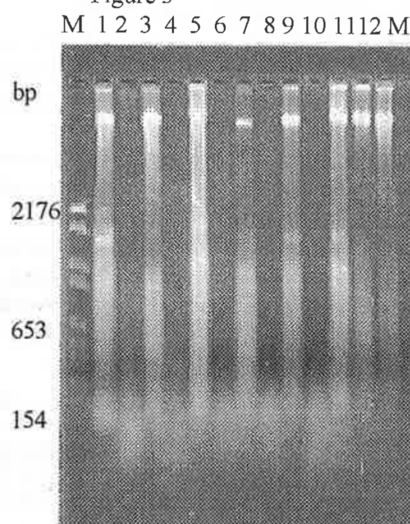
Typical RAPD profiles showing identical types from duplicate enrichments (lanes 3 and 4 and lanes 7 and 8)

Figure 2



Typical *Fla A* PCR/RFLP types from retail chicken survey. Lanes 5,6 and 7 show untypable isolates.

Figure 3



Stable Nucleon extracted DNA is shown in lanes 1,3,5,7,9,11,12 and 13. All other lanes show unstable CTAB extracted DNA after storage.

M - Molecular weight Marker which was DNA (pBR 328) digested with *Bgl* 1 and *Hinf* 1 (Boehringer Mannheim).