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Genotypic analysis of *Escherichia coli* recovered from product and equipment at a beef-packing plant

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ABSTRACT

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Aims: To identify sources of *Escherichia coli* on beef by characterizing strains of the organism on animals, equipment and product at beef-packing plant.

Methods and Results: Generic *E. coli* were recovered from hides, carcasses, beef trimmings, conveyers and ground beef during the summer of 2001 (750 isolates) and winter of 2002 (500 isolates). The isolates were characterized by Random Amplification of Polymorphic DNA (RAPD). The numbers of *E. coli* recovered from dressed carcasses were less than the numbers recovered from hides. The numbers recovered from chilled carcasses were too few for meaningful analysis of the strains present on them but the numbers recovered from trimmings and ground beef were larger. The RAPD patterns showed that the majority of isolates from hides, carcasses, beef trimmings, conveyers and ground beef were of similar RAPD types, but a few unique RAPD types were recovered from only one of those sources. The *E. coli* populations present on the hides of incoming animals and in the beef-processing environment were highly diverse. Randomly selected *E. coli* isolates from each of the five sources were further characterized by pulsed-field gel electrophoresis (PFGE). Most genotypes of *E. coli* defined by PFGE corresponded to the *E. coli* types defined by RAPD.

Conclusions: The hides of the incoming animals appeared to be only one of the sources of the *E. coli* on trimmings and in ground beef, as additional sources were apparently present in equipment used for carcass breaking.

Significance and Impact of the Study: This study indicates that hazardous microbiological contamination of meat may occur after the dressing of carcasses at commercial beef-packing plants, which suggests that attention should be given to the control of the contamination of meat during carcass breaking as well as during the dressing of carcasses.

Keywords: beef, *E. coli*, packing, RAPD-typing.

INTRODUCTION

Pathogenic *Escherichia coli* particularly serotype O157:H7 and other Shiga toxin-producing *E. coli* (STEC) strains have been responsible for outbreaks of enteric and systemic disease associated with the consumption of undercooked ground beef (Tuttle *et al.* 1999). Cattle are thought to be the main reservoir of pathogenic *E. coli*, as between 10 and 80% of cattle have been reported to carry STEC (Beutin *et al.* 1993).

Escherichia coli are transferred from animal hides onto carcasses during carcass dressing processes (Bell 1997; Elder *et al.* 2000). A correlation between identical molecular subtypes of *E. coli* O157:H7 present in the faeces of cattle

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and those isolated from hides and carcasses during slaughter and processing has indicated a direct link between the *E. coli* found in animals and on the carcasses derived from them (Barkocy-Gallagher *et al.* 2001; Bonardi *et al.* 2001). However, the relationship between the *E. coli* found on skinned carcasses and those found on meat at later stages of processing is uncertain. This relationship must be defined for proper understanding of the sources of hazardous microbiological contamination of ground beef.

The small numbers of pathogenic *E. coli* found on meat limits the possibilities for unambiguously identifying the sources of such contaminants. However, sources of possible hazardous microbial contamination may be identified by determining the sources of generic *E. coli*, which is an acceptable indicator for the possible presence of enteric pathogens (Brown *et al.* 2000).

Data have been presented which show that decontaminating treatments applied to beef carcasses at North American packing plants can essentially eliminate *E. coli* from the meat (Arthur *et al.* 2002). Despite that many continue to assume that enteric pathogens in ground beef must be directly derived from contaminants deposited on carcasses during carcass dressing (Elder *et al.* 2000; Barkocy-Gallagher *et al.* 2001). Others have suggested that carcass-breaking equipment may be implicated in the contamination of beef (Gill and McGinnis 2000). Direct evidence to confirm either hypothesis is lacking.

Data obtained by cultural methods can demonstrate quantitative changes in meat microflora but they cannot provide conclusive information about the relatedness of the strains, or the sources of contamination. A more discriminatory approach in identifying the sources of microbial contaminants in beef-packing plants has involved the molecular typing of bacterial strains. Molecular typing methods such as enterobacterial repetitive intergenic consensus-PCR, random amplification of polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) have been used to identify the sources of pathogens in meat-processing environments (Giovannacci *et al.* 1999; Galland *et al.* 2001; Geornaras *et al.* 2001; Bouvet *et al.* 2002a,b; Warriner *et al.* 2002; Tutenel *et al.* 2003). These methods allow genetically related strains recovered from different sources to be recognized, which can indicate the possible sources of meat contaminants. Using such methods, generic *E. coli* has been traced from cattle slaughtered at a research abattoir to ground beef produced from the carcasses (Aslam *et al.* 2003). Processing at the research abattoir did not reflect the complexity of commercial beef processing at a plant where slaughtering is at the rate of 250 cattle per hour. Therefore it was logical to extend the methods developed under controlled experimental conditions to a commercial plant to obtain information on the sources of *E. coli* contamination.

MATERIALS AND METHODS

Sample collection

Samples were collected at a commercial beef-packing plant where carcasses are processed at a rate of 250 carcasses per hour. Samples were obtained during each of the summer of 2001 and winter of 2002, with samples being collected within a period of 60 days during each season. The individual animals were not followed throughout the process as it was impracticable in the plant where 250 animals are slaughtered per hour. There was no relationship between the various samples collected during one visit and animals slaughtered in the summer and in winter. The sampling protocols used in this study have been published in previous reports (Gill *et al.* 1999, 2001; Gill and Jones 2000). The sources of samples were the hides of carcasses before skinning, dressed carcasses before they were pasteurized, carcasses leaving the carcass chiller, conveyers for carcass portions or meat cut, trimmings from carcass portions and cuts, and ground beef prepared from the trimmings (Fig. 1). During each visit, only five samples were collected from any one source. Sampled product was selected at random. Between 25 and 40 samples were collected from each source during each season. Samples were obtained from approx. 100 cm^2 areas of the hides, beef trimmings and conveyers; 1000 cm^2 areas of washed carcasses; and from the whole of the distal surface of each chilled side (approx. 12 500 cm^2). Each sample from carcasses or conveyers was obtained by swabbing the surface with a 5×5 cm, 8 ply, sterile gauze swab (Curity gauze sponge, Kendall Canada Inc., Peterborough, ON, Canada) which had been moistened with 0·1% w/v peptone water (Becton Dickinson Co., Sparks, MD, USA). Samples from the conveyers were obtained from drive mechanisms, rollers and idlers after routine cleaning and before the start of processing. Samples from beef trimmings were obtained by swabbing the meat with sterile, 3×4 cm cellulose acetate sponges (Speci-sponge, Nasco Canada, Aurora, ON, USA) moistened with peptone water. Ground beef samples (200 g) were obtained from 4·5-kg chub packs of coarsely ground meat prepared at the plant.

Isolation and identification procedures

Each swab or sponge sample was pummelled with 10 ml of sterile peptone water in a stomacher (Stomacher 400 Lab Blender, Seward Medical, London, UK) for 2 min. Serial 10-fold dilutions of the stomacher fluid were prepared in peptone water. Ground beef samples were processed by homogenization and centrifugation, as described previously (Aslam *et al.* 2003).

The entire volume (9 ml) of undiluted stomacher fluid and/or 9 or 10 ml of appropriate dilutions of each sample

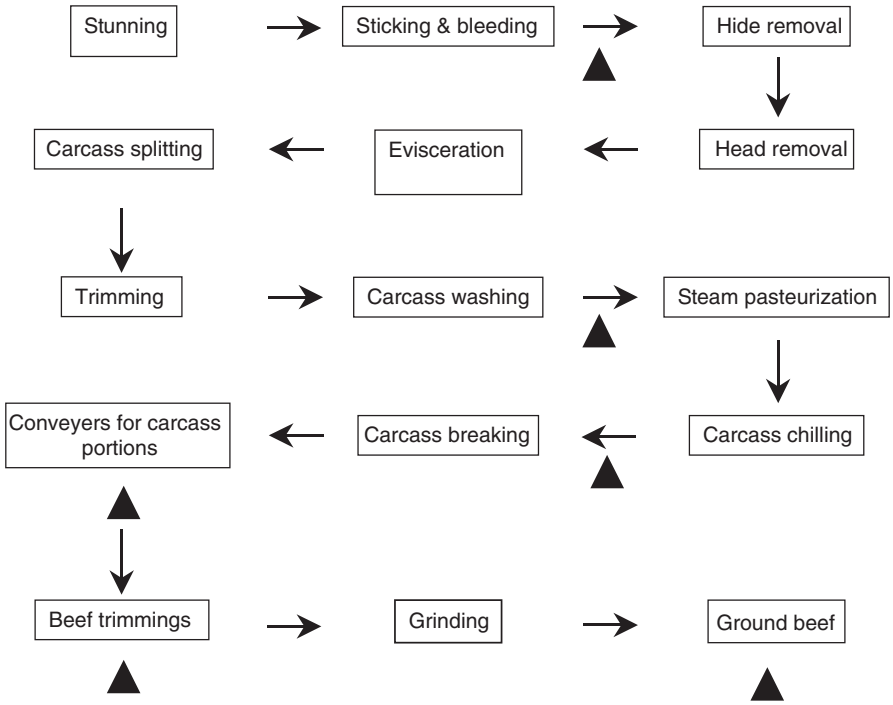

Fig. 1 Schematic flow diagram of beef-packing plant. Arrowheads indicate sample sources

were filtered through a hydrophobic grid membrane filter (QA Life Sciences Inc., San Diego, CA, USA). Filters were placed on SD-39 agar (Oxoid Inc., Nepean, ON, Canada). The plates were incubated at 42°C for 18 h. Square containing green colonies were counted and a most probable number for presumptive *E. coli* was obtained from that count (Entis and Lerner 1997). All bacterial counts were converted to $\log_{10}$ values and the mean $\log_{10}$ and standard deviation for each set of counts were calculated. Twenty-four randomly selected colonies from each filter were subcultured on SD-39 agar and incubated at 42°C for 18 h. An isolated colony was picked from each plate and was suspended in a PCR tube containing 100 μl of sterile water for preparation of a DNA template. Not all recovered isolates were subjected to RAPD analysis.

Confirmation of generic *E. coli*

Fifty randomly selected *E. coli* isolates from each of source except chilled carcasses were confirmed as generic *E. coli* using a PCR assay targeting the universal stress protein (*uspA*) gene, as described by Chen and Griffiths (1998).

RAPD analysis of *E. coli* isolates

Only 150 and 100 isolates obtained from each of the sources except chilled carcasses during summer and winter, respectively were used. Each of these isolates was analysed by the RAPD method, as previously described (Aslam *et al.* 2003). The numbers of *E. coli* recovered from chilled carcasses were too few to include in RAPD analysis for meaningful comparison of data.

For each RAPD analysis, the 25-μl PCR mixture contained 5 μl of template DNA, 2·5 mM $MgCl_2$, 0·8 nM 1254 decamer primer (5′-CCGCAGCCAA-3′; Sigma-Aldrich, Canada Ltd, Oakville, ON), 300 μM of each deoxynucleoside triphosphate (Invitrogen Corporation, Carlsbad, CA, USA) and 1 U *Taq* DNA polymerase (Sigma-Aldrich). PCR was performed in a Robocycler Infinity Thermocycler (Stratagene Inc., La Jolla, CA, USA) using the amplification and cycling protocol described previously (Aslam *et al.* 2003). Appropriate positive and negative controls were included during each RAPD analysis. Amplified DNA fragments were separated on 1·8% agarose gel (90 V for 2 h) and stained with ethidium bromide (0·25 μg ml^{-1}) for 45 min. A 100-bp DNA ladder (Invitrogen Corporation) was included on the gel as a size marker.

PFGE analysis of *E. coli* isolates

After the RAPD analysis of 750 isolates from summer samples, 48 isolates were selected to represent a range of RAPD types from each of the five sample sources. PFGE analysis of these isolates was performed by the standard method of the Centers for Disease Control and Prevention (1998).

Analysis of DNA patterns

Stained agarose gels were scanned digitally with a Kodak EDAS290 system (Eastman-Kodak, Rochester, NY, USA) and images were stored as tagged image format files. DNA patterns were analysed with Molecular Analyst Software, Fingerprinting version 1·61 (Bio-Rad Laboratories, Hercules, CA, USA), after conversion and normalization. Similarities between the DNA patterns based on band positions were determined from the Dice similarity coefficients calculated by software. The RAPD patterns with Dice similarity coefficients greater than 0·8 were considered genetically related and were assigned an RAPD-type. The DNA patterns obtained with *XbaI* PFGE typing of *E. coli* isolates were also compared using Dice similarity coefficient.

The RAPD patterns of *E. coli* isolates obtained from pairs of sources during the same season were compared, to identify the shared (similar RADP patterns) and unique RAPD types. For this analysis 150 and 100 isolates obtained from each source in summer and winter, respectively, were examined.

In addition, the RAPD patterns of *E. coli* isolates obtained from all sources during each season were compared to identify the RAPD types that were common to all sources. RAPD types unique to one source within a season were also identified through this analysis.

RESULTS

E. coli counts

Mean $\log_{10}$ numbers of *E. coli* recovered from hides were >1$\log_{10}$ unit more in summer than in winter (Table 1). In both summer and winter the mean $\log_{10}$ numbers recovered from washed carcasses were <1$\log_{10}$ CFU 1000 cm^{-2}, and *E. coli* were recovered from <10% of chilled carcasses. *E. coli* were recovered from the majority of samples from beef trimmings, all samples of ground beef, and from ≤52% of the samples from conveyers. For all sources the numbers of *E. coli* recovered were higher during the summer than the winter months. All 50 randomly selected isolates from each of those sources were confirmed as generic *E. coli*.

Sources and genetic diversity of *E. coli*

The numbers and percentages of *E. coli* isolates that were shared between two sources in pairs of all sources except chilled carcasses are shown in Table 2. For samples collected in summer, most of the isolates from each of the other sources belonged to RAPD types that were found in ground beef also. For samples collected in winter, <50% of

Table 2 Numbers and percentages of *E. coli* isolates recovered during summer and winter from various sources in a beef-packing plant that were RAPD types common to both of two sources

Source A vs. source B		Isolates of common RAPD types [*n* (%)]	
		Summer*	Winter†
Ground beef	Hides	243 (81)	85 (43)
	Carcasses	250 (83)	132 (66)
	Beef trimmings	257 (86)	115 (58)
	Conveyers	180 (60)	76 (38)
Hides	Carcasses	245 (81)	91 (46)
	Beef trimmings	233 (78)	91 (46)
	Conveyers	158 (52)	80 (40)
Carcasses	Beef trimmings	246 (82)	92 (46)
	Conveyers	161 (53)	46 (23)
Beef trimmings	Conveyers	188 (62)	83 (42)

*150 isolates/source characterized by the RAPD method.
†100 isolates/source characterized by the RAPD method.

Table 1 Number of samples yielding *E. coli*, and mean and standard deviation (S.D.) of numbers of *E. coli* recovered during summer and winter at a commercial beef-packing plant

Sample sources	Summer sampling			Winter sampling		
	Mean	S.D.	*n*¶/*N***	Mean	S.D.	*n*/*N*
Hides*	3·94	0·90	30/30	2·64	1·10	28/30
Washed carcasses†	0·59	0·70	31/40	0·25	0·70	26/40
Chilled carcasses‡	−0·47	0·46	3/35	−0·32	0·19	3/40
Beef trimmings*	0·89	0·70	30/35	0·59	1·10	24/40
Ground beef§	1·72	0·70	25/25	1·34	0·50	25/25
Conveyers*	0·97	1·60	13/25	0·20	1·20	10/30

*$\log_{10}$ CFU 100 cm^{-2}.
†$\log_{10}$ CFU 1000 cm^{-2}.
‡$\log_{10}$ CFU recovered from the whole distal surface of each carcass (approx. 12 500 cm^2).
§$\log_{10}$ CFU 200 g^{-1}.
¶Number of samples from which *E. coli* were isolated.
**Total number of samples.

the isolates recovered from ground beef belonged to RADP types found on hides and/or conveyers, but >50% of the isolates from ground beef belonged to RAPD types found on washed carcasses and/or beef trimmings.

The 750 *E. coli* isolates obtained during summer were grouped into 102 RAPD types and the 500 isolates obtained during winter were grouped into 115 RAPD types. For samples collected in summer, 38 RAPD types comprised 605 isolates that were recovered from beef trimmings and ground beef were also found in samples from the hides, washed carcasses and conveyers (Table 3). Of those 38 RAPD types, 28 were common to ground beef and one or more, but not all other sources, and nine types were found in samples from all sources. The majority of isolates from all sources belonged to the predominant RAPD types 11, 17, 38 and 40. Fewer isolates of the same RAPD types were recovered from both ground beef and samples from other sources during winter.

No *E. coli* isolate of unique RAPD-type was found in the ground beef samples obtained during the summer, but few isolates of unique RAPD types were recovered from each of hides (9% isolates), washed carcasses (11% isolates), beef trimmings (5% isolates) and conveyers (27% isolates). During the winter, some *E. coli* isolates (11%) of unique RAPD types were recovered from ground beef only, and *E. coli* isolates unique only to the hides (25% isolates), washed carcasses (9% isolates), beef trimmings (18% isolates) and conveyers (29% isolates) were also found. It was found that 11 and 21% of isolates in summer and winter, respectively, belonged to RAPD types that were found on carcasses, beef trimmings, and conveyers and in the ground beef but not on hides.

Table 3 *Escherichia coli* RAPD types that were recovered at various stages of processing in a beef-packing plant

	No. of isolates*					
RAPD types	H†	WC	CE	BT	GB	Total isolates
6	8	1	1	11		21
10	5	1	1	1	1	9
11	14	7	13	5	29	68
12	1	3	1	3		8
13	11	1	1	1		14
15	8	1		2		11
17	36	14	17	24	48	139
18	1		1	2	2	6
19			3		1	4
20	1	1			1	3
21			8	2		10
23		3		1	1	5
25	6	1	1	4	1	13
26			1	3		4
27			1		1	2
29	2	5		4	1	12
30		5		2	1	8
31				1	1	2
32	2	2	2	6		12
33				1	3	4
34		1			3	4
35	2	1	1	3	2	9
37		1		1	1	3
38	9	8	11	9	31	68
39		4		8	3	15
40	2	13	5	9	4	33
43			1	5	2	8
47	1	2		5	1	9
48	1	2		1		4
50	3	4		6		13
51			2	2		4
52			1	1	1	3
62	3	8	1	2	2	16
63		1	1		1	3
64	1	28		1	3	33
65		2		1	1	4
71			13		1	14
74	3	1	1	1	1	7

*150 isolates/source characterized by the RAPD method.
†Sample sources: H, hides; WC, washed carcasses; CE, conveyers; BT, beef trimmings; GB, ground beef.

PFGE analysis of *E. coli* isolates

Fifty-four per cent of *E. coli* isolates were similarly grouped by either PFGE or RAPD, and 33% of the isolates were identified as unique by both. Remaining 13% of the isolates were indistinguishable by the RAPD method but could be differentiated by PFGE. To exemplify those relationships Fig. 2 shows dendrograms depicting PFGE and RAPD patterns of 12 isolates obtained from various sources. The two isolates in each of the pairs 21 and 30 and 22 and 33 were indistinguishable by either method. Isolates 34 and 36 were found to be distantly related by PFGE but were closely related by the RAPD method. The dendrograms for both PFGE and RAPD analysis showed that isolates 25, 26 and 39 were only distantly related.

DISCUSSION

In the present study, the numbers of *E. coli* recovered from various sources in a beef-packing plant were comparable with numbers previously found at similar sources (Hardin *et al.* 1995; Bell 1997; Gill and McGinnis 2000; Aslam *et al.* 2003).

The PFGE is used as a gold standard in epidemiological investigations to determine if disease-causing strains match those isolated from suspected sources (Beutin *et al.* 1997; Pradel *et al.* 2001). Thus, the rationale for using PFGE in the present study was to establish the validity of RAPD

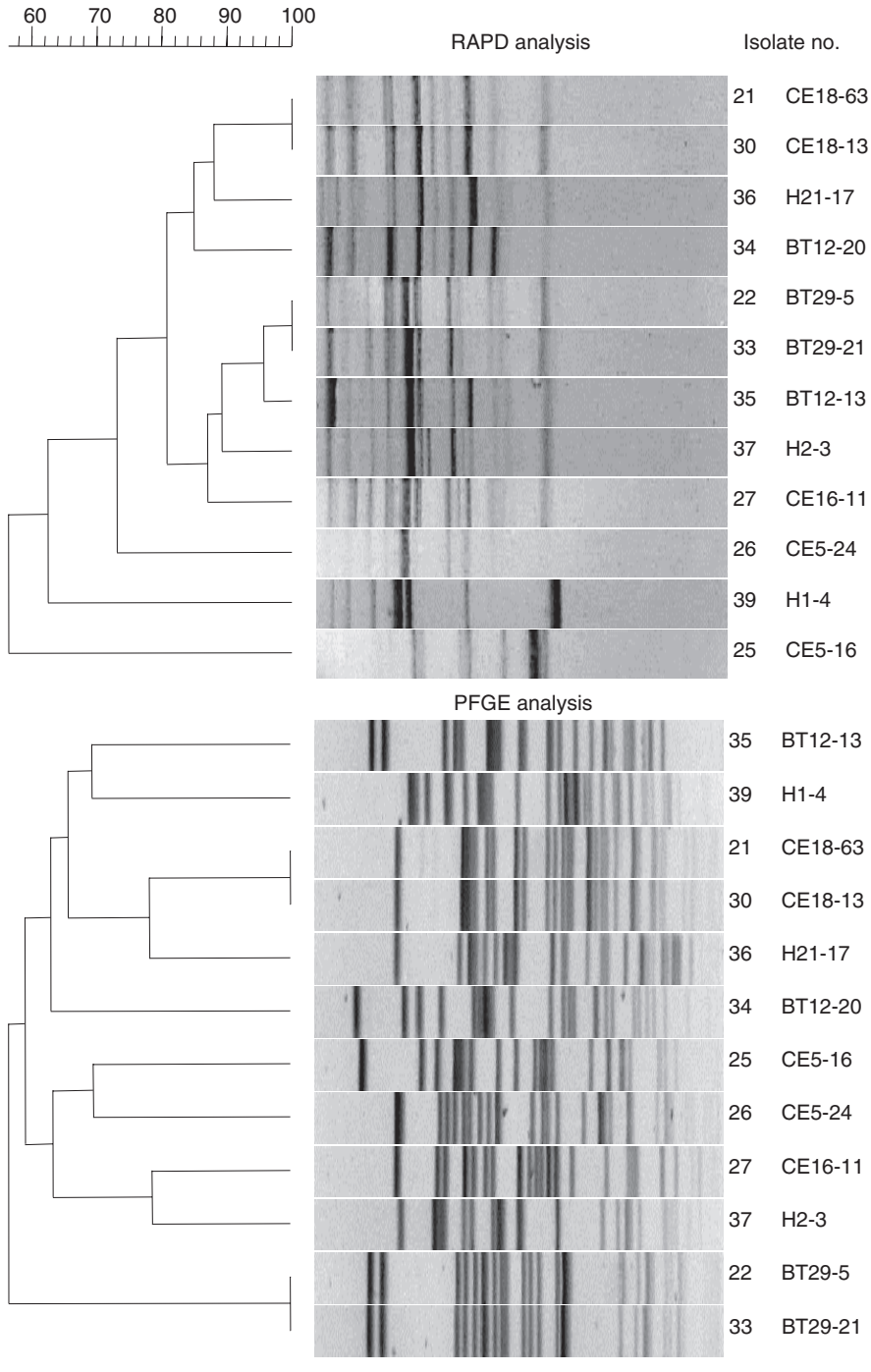

Fig. 2 Dendrograms obtained from RAPD and PFGE analysis of *E. coli* isolates recovered from various sources in a beef-packing plant. H, hide; BT, beef trimmings; CE, conveyers

typing. A good correlation between the results obtained with both methods confirmed that the RAPD method was a valid approach in differentiating the isolates recovered in this study. The majority of isolates were differentiated into corresponding genetic types by both RAPD and PFGE, which is consistent with the findings of a previous study (Khan *et al.* 2002). However, a few isolates were characterized into multiple PFGE types whereas these same isolates grouped into as a single RAPD-type. The greater discriminatory power of PFGE has been previously reported (Grif *et al.* 1998; Khan *et al.* 2002). The molecular mechanism involved in the PFGE and RAPD methods are different. With RAPD strains are differentiated on the basis of randomly selected portions of genomic DNA where PFGE identifies genetic differences due to the presence of restriction digestion sites in the whole of the genomic DNA. This

accounts for some of the differences in results obtained with PFGE or RAPD. In practice the RAPD is often preferred, because the PFGE technique is laborious and less economical when a large number of isolates must be characterized in a short time as in this study.

The majority of the *E. coli* isolated from the hides of incoming animals, carcasses and conveyers were genetically related to those isolated from ground beef. The recovery of few unique strains in ground beef suggests that most of the contaminants in ground beef originated from the processing steps before grinding. The source of the few unique RAPD types of *E. coli* isolated from ground beef prepared during the winter months might have been the grinding equipment. Previous studies have suggested that inadequate cleaning of meat grinders can result in the contamination of ground beef (Farrell *et al.* 1998) despite the fact that cleaning is deemed adequate by most regulatory criteria.

A few RAPD types of *E. coli* were recovered from the ground beef, washed carcasses, beef trimmings and conveyers, but not from the hides of animals. This suggests that some *E. coli* in ground beef are derived from the plant environment. Previous studies conducted in pig- and poultry-processing plants using molecular techniques have suggested a link between the pathogens found on live animals and those found on product and equipment (Geornaras *et al.* 2001; Giovannacci *et al.* 2001; Bouvet *et al.* 2002a; Warriner *et al.* 2002; McEvoy *et al.* 2003). The hides of incoming animals might not directly contribute *E. coli* to ground beef as no RAPD-type of *E. coli* was recovered from only ground beef and hides. However, an indirect role of the hide in spreading *E. coli* throughout the plant seems likely, as most RAPD types from the hides were recovered from all sources.

In previous studies it has been shown that cooled carcasses entering some carcass-breaking facilities were largely free of *E. coli*, but that substantial numbers of *E. coli* were present on cleaned conveyers, beef trimmings and cuts and in ground beef in those facilities, which suggested that conveyers were a likely source of the *E. coli* on the meat (Gill *et al.* 1999, 2001; Gill and McGinnis 2000). In this study, the findings with regard to number of *E. coli* were similar. However, the majority of isolates recovered from dressed carcasses, beef trimmings and ground beef were genetically related, which indicates a common source of contamination for all of these sites. It is quantitatively impossible for the *E. coli* found in the breaking facility to be directly derived from incoming carcasses as there is no direct passage between the area of cattle slaughtering and carcass-breaking facility. Therefore *E. coli* contamination must be brought in by other means and/or be brought in on carcasses and grow within the facility.

The relatively numerous unique isolates found only on the conveyers suggests that these sites were continuously contaminated with low numbers of *E. coli* over a period of time, and that those organisms form a resident population of *E. coli*. Persistent populations of *E. coli* growing on inadequately cleaned equipment may therefore be the proximate sources of most of the *E. coli* found in ground beef. However, the dominant clones in *E. coli* populations within animals change genetically continuously over time (Jarvis *et al.* 2000). Similar changes in resident populations on equipment might then be expected possibly due to the stress imposed by low temperature in the processing environment and the sanitizers applied. Investigation of that matter would seem desirable.

Genetic diversity among *E. coli*, particularly pathogenic strains has been previously reported (Jarvis *et al.* 2000; Galland *et al.* 2001; Pradel *et al.* 2001). The RAPD patterns data in the present study suggested that genetically related *E. coli* types were shared among various sources despite the presence of a few unique RAPD types. These results corroborate previous findings which reported that highly diverse strains of *E. coli* can be found during slaughter and meat processing (Barkocy-Gallagher *et al.* 2001; Arthur *et al.* 2002; Bouvet *et al.* 2002a; Warriner *et al.* 2002). The genetic diversity observed in the *E. coli* isolated from the beef-processing environment probably reflects the high degree of genetic diversity observed in *E. coli* in both pasture and feedlot cattle (Faith *et al.* 1996; Galland *et al.* 2001; Aslam *et al.* 2003; Renter *et al.* 2003).

In conclusion, this study has demonstrated that *E. coli* found on the hides of incoming cattle as well as in the beef-packing environment is responsible for ground beef contamination. The conveyers are also a source of ground beef contamination and there is considerable opportunity for cross-contamination among various sources in the packing plant. Molecular typing methods provide a valuable tool for identifying the sources of contamination during beef processing and a combination of RAPD, used as a screening tool, followed by more detailed analysis using PFGE would be a good approach for studies of this type. Molecular characterization of generic *E. coli* has demonstrated that this organism is genetically diverse. The results of this study would further suggest that research should be focused on improving fabrication hygiene and equipment cleanliness in addition to the microbiological condition of carcasses.

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