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Cross-contamination of carcasses and equipment during pork processing

K. Warriner, T.G. Aldsworth, S. Kaur and C.E.R. Dodd
Division of Food Sciences, School of Biosciences, University of Nottingham, Loughborough, Leics., UK

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K. WARRINER, T.G. ALDSWORTH, S. KAUR AND C.E.R. DODD. 2002.
Aims: The cross-contamination events within a commercial pork processing line were examined by a combination of ERIC-PCR DNA fingerprinting of *Escherichia coli* and plate counts.
Methods and Results: Sponge sampling of environmental surfaces and carcasses was performed over an 8-h processing period. Prior to the start of processing the scraper and dry polisher blades were found to harbour substantial Enterobacteriaceae and *Escherichia coli* populations. From plate count data the key cross-contamination site for the transfer of bacteria between carcasses occurred during evisceration. However, DNA fingerprints of representative *E. coli* isolates identified that genotypes initially present on the scraper/dry polisher became distributed on wet polisher blades, band-saw and butcher's hands despite a singeing step being performed post dry polishing. A high proportion of *E. coli* on post-eviscerated carcasses could be traced to down-stream (pre-singe) environmental contact surfaces.
Conclusions: DNA fingerprinting has demonstrated that *E. coli* and potential enteric pathogens can be transferred between pork carcasses throughout the processing line. In this respect scalding and singeing cannot be relied upon to control cross-contamination of enteric bacteria between carcasses.
Significance and Impact of the Study: Sole reliance on indicator organism counts to identify cross-contamination events as currently advocated is limited.

INTRODUCTION

In the process of pig slaughter a wide range of potential pathogens, such as *Salmonella* spp. (Currier *et al.* 1986; Borch *et al.* 1996; Berends *et al.* 1997; Korsak *et al.* 1998), *Listeria monocytogenes* (Gill and Jones 1995; Nesbakken *et al.* 1996; Autio *et al.* 2000), amongst others (Akier *et al.* 1989; Duffy *et al.* 2001), can contaminate the surface of carcasses. Although there are many opportunities for carcass contamination to occur during slaughter, the main emphasis of control is applied at the end of evisceration in the form of washing. Nevertheless, the initial scalding and singeing steps that are performed to de-hair carcasses have also been demonstrated to remove a substantial proportion of the carcass surface microflora (Sorquist and Danielssen 1986; Borch *et al.* 1996) and can be considered to act as barriers to minimize the transfer of pathogens through the line. However, for more effective control of pathogen spread there is a need to develop a hazard analysis critical control point scheme within the pig slaughter process. Central to implementing such a programme is to identify key unit operations implicated in cross-contamination, thereby enabling control measures to be applied.

Many reports have been published that highlight the potential for carcass contamination during de-hairing and evisceration operations (Gill and Bryant 1993; Nesbakken *et al.* 1994; Rivas *et al.* 2000; Yu and Palumbo, 2000). Such studies have been based on enumerating total aerobic and indicator organism counts from samples recovered from carcasses. However, although such methods permit the gross changes in carcass microflora to be determined this does not provide sufficient data to elucidate the origins of pathogens. In addition, as pathogens typically occur in low numbers, contamination of carcasses is not necessarily reflected by an increase in bacterial counts. A more sensitive approach to study cross-contamination events is by applying molecular

Correspondence to: Dr C.E.R. Dodd, Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leics. LE12 5RD, UK (e-mail: christine.dodd@nottingham.ac.uk).

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typing techniques (Dodd 1988; Dodd 1994). Molecular typing has previously been applied in epidemiology studies tracing the origins of *Listeria monocytogenes* (Giovannacci *et al.* 1999; Autio *et al.* 2000) and *Salmonella* (Giovannaci *et al.* 2001) within pork slaughter lines. However, due to the low level and sporadic occurrence of pathogens the direct identification of cross-contamination events is problematic. In contrast, *Escherichia coli* is widely distributed within the slaughterhouse and, although not necessarily pathogenic, can be used to indicate faecal contamination, hence the potential transfer of enteric pathogens. Therefore, by following the distribution of *E. coli* types within slaughter lines a more definitive assessment of cross-contamination events can be established.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR provides a rapid and versatile means of differentiating related strains of a diverse range of bacteria (Versalovic *et al.* 1991; Hermans *et al.* 1995; Rivera *et al.* 1995; Marty 1997; Davin-Regli *et al.* 1998; Sciacchitano 1998) including *E. coli* (Osek 1999). In the following study ERIC-PCR has been applied to determine the distribution pattern of *E. coli* types within a pig slaughter line during processing. This, in combination with bacterial counts, has been applied to identify the transfer of bacteria between carcasses and contact surfaces during processing, thereby enabling identification of critical control points in pork production.

MATERIALS AND METHODS

Sample collection

Sampling was performed within a United Kingdom slaughterhouse processing 350 pig carcasses per hour. The plant was designed with physical separation of sticking, de-hairing and evisceration operations (Fig. 1). After bleeding, the pigs were scalded in a water tank for 5 min at 69 °C to loosen surface hair. The post-scald carcasses were transferred to a scraper unit that consisted of a rotating drum lined with coarse brushes that removed gross organic matter and hair from the skin. The pigs were then removed from the line to a table where the toe-nails were removed. Carcasses were then returned to the line where the residual hair on carcasses was removed by a combination of dry polishing, singeing and wet polishing. The dry polisher consisted of four vertical opposing banks of rotating flanges/blades through which the carcasses passed. Carcasses were then singed in an open cabinet with two sets of flame banks (residence time *ca* 6 s). Immediately following singeing the carcasses traversed through a wet polishing unit that was of the same construction as the dry polisher save that a continuous water spray showered the carcasses. The de-haired carcasses were then transferred to the evisceration area via overhead rails. The post-eviscerated carcasses were halved and subsequently washed using a pressure hose prior to being transferred to a chill room.

Prior to the start of processing, samples derived from scald water, halving power saw, blades of the scraper, dry polisher and wet polisher were taken. The same sites were also sampled at the mid-point and end of processing. Due to restricted access to a number of unit operations, carcass sampling was performed at pre-scald, pre-dry polishing, pre- and post-evisceration (Fig. 1). Random carcasses (10) were sampled at each designated point on the line at *ca* 45-min intervals. Sampling was performed using Polywipe sponges pre-moistened in 0·1% peptone water containing 0·1% v/v Tween-80 (Medical Wire & Equipment, Bath, UK). Sterile aluminium templates (10 × 10 cm) were used to designate an area on the carcass brisket for sponge sampling. The samples were placed in sterile stomacher bags and maintained at 4 °C until required for microbiological examination.

Microbiological analysis

Bacteria were released from the sponges by the addition of 30 ml buffered peptone water (Oxoid, Basingstoke, UK) and stomaching for 2 min. Serial dilutions were prepared in maximum recovery diluent (Oxoid, UK). Total aerobic counts were enumerated on plate count agar (Difco, MI, USA) incubated at 30 °C for 48 h. Enterobacteriaceae numbers were determined using Violet Red Bile Glucose Agar (Oxoid, UK) that was incubated at 30 °C for 48 h. *Escherichia coli* were enumerated using *E. coli* Petri film (3M, MN, USA) that was incubated at 37 °C for 24 h.

DNA fingerprinting

Preparation of DNA. Extraction of DNA from *E. coli* isolates was performed according to the method described by Polysou *et al.* (2000). A maximum of 10 representative *E. coli* colonies from each sample were grown overnight on Luria Bertani agar (Difco, USA) at 37 °C. A colony of *E. coli* was dispersed into 0·9 ml sterile distilled water containing 0·1 ml 35% v/v formaldehyde (to inactivate nucleic acid degrading enzymes). The suspension was incubated at room temperature for 1 h and the cells subsequently harvested by centrifugation (13 000 × *g* for 90 s). The cell pellet was washed three times in sterile MRD and finally resuspended in 0·2 ml TE buffer. Cell lysis was achieved by heating the suspension in a boiling water bath for 10 min. Cell debris was removed by centrifugation (13 000 × *g* for 10 min) and the supernatant containing the DNA decanted into a sterile eppendorf tube.

ERIC-PCR. The *E. coli* isolates were typed using ERIC-PCR (Versalovic *et al.* 1991). The primers used were:

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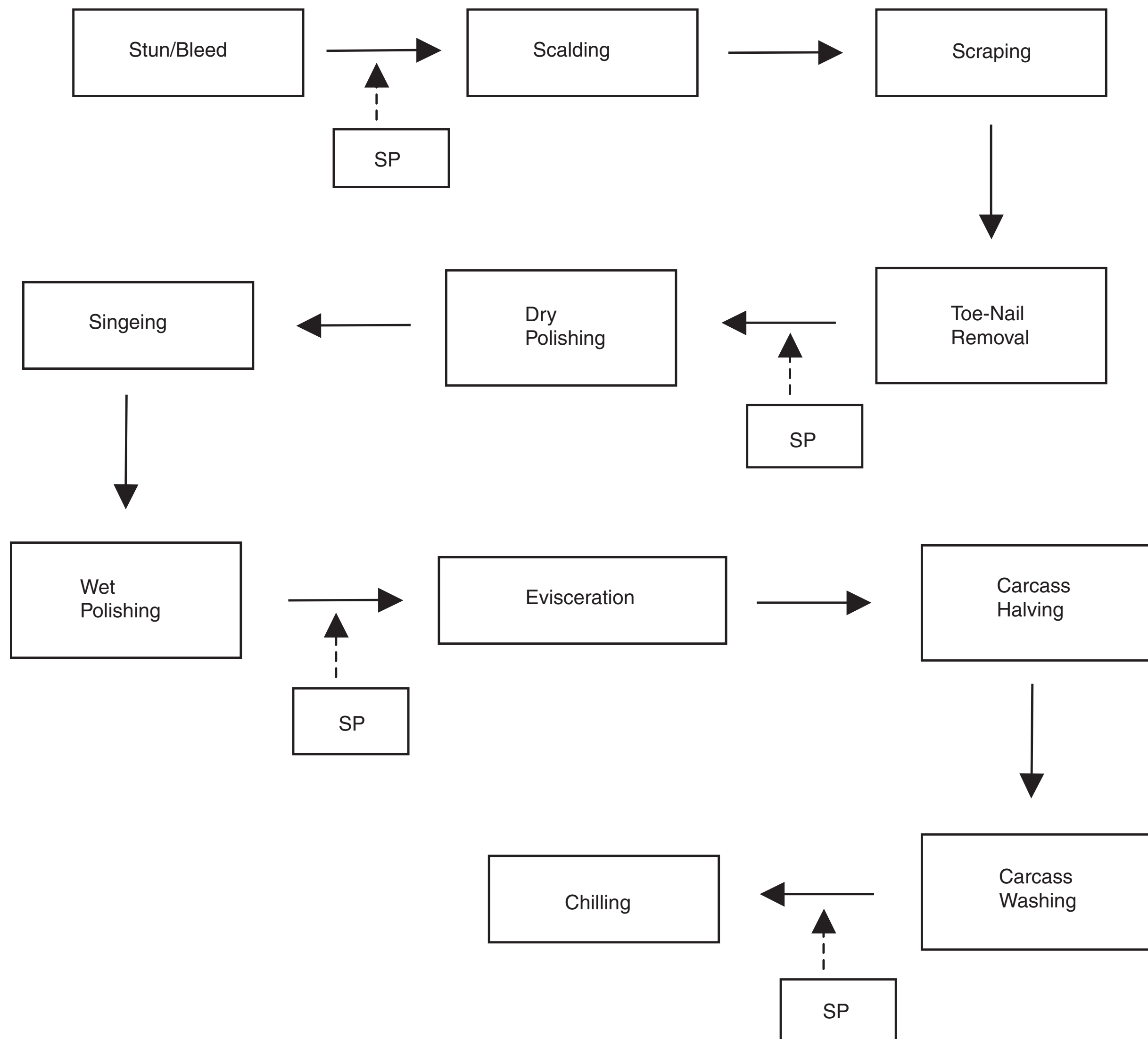

Fig. 1 Schematic flow diagram of the pig slaughter line. SP = Sampling point

ERIC1 (forward) 5′-ATGTAAGCTCCTGGGGATT-CAC-3′; ERIC2 (reverse) 5′-AAGTAAGT-GACTGGGGTGAGCG-3′.

The PCR reactions were carried out in a total reaction volume of 25 μl. A 1 μl DNA sample was added to 24 μl master mix containing (final concentration): 100 pmol l^{-1} of each primer (Sigma-Genosys, Poole, UK), 1 U of *Taq* DNA polymerase (Advanced Biotechnologies, Surrey, UK), 0·2 mmol l^{-1} each of the deoxribonucleotide triphosphates dATP, dCTP, dGTP and dTTP (Promega, Madison, USA), 4 mmol l^{-1} $MgCl_2$ (Advanced Biotechnologies, UK), and reaction buffer (50 mmol l^{-1} Tris-HCl pH 9·2 containing 14 mmol l^{-1} $(NH_4)_2SO_4$, 2% v/v DMSO and 0·1% v/v Tween 20). The reactions were carried out in a Progene thermocycler (Techne, Cambridge, UK) at the following temperatures: one cycle for 3 min at 94 °C, then 35 cycles comprising 30 s at 94 °C, 1 min at 52 °C, 4 min at 65 °C. The final cycle was for 8 min at 65 °C.

The PCR product was mixed with 5 μl loading buffer and electrophoresed on a 2% w/v agarose gel, containing ethidium bromide, 0·5 μg ml^{-1}, in TE running buffer at 70 V for 2 h (Versalovic *et al.* 1991). Comparison of the DNA fingerprints was undertaken using Amersham Pharmacia, ImageMaster® 1D-Elite gel analysis and database software (Amersham Pharmacia Biotech, Bucks., UK). Dendrograms were constructed using the Dice similarity coefficient (S_D) and the UPGMA (unweighted pair group method using arithmetic averages) clustering algorithm (Sokal and Sneath 1963). Major clusters were formed at the 70% similarity level.

RESULTS

Total aerobic counts, Enterobacteriaceae and *Escherichia coli* counts on carcasses during processing

Random sampling of carcass briskets was performed at specified time intervals at different points in the processing line (Fig. 1) throughout an 8-h period of activity. The total aerobic counts recovered from pig carcasses did not differ significantly ($P > 0{\cdot}10$) throughout the period of activity or at the different points sampled in the processing line (Table 1).

The Enterobacteriaceae counts on pre-scald carcasses remained essentially constant throughout the period of activity ($2{\cdot}00 \times 10^4 \pm 57$ cfu 100 cm^{-2}). Enterobacteriaceae counts on carcasses that had undergone scalding, scraping and toe-nail removal (pre-dry polishing) generally decreased to a level of $5{\cdot}01 \times 10^3 \pm 4$ cfu 100 cm^{-2}; that remained unchanged throughout the processing period.

With carcasses that had undergone dry polishing, singeing and wet polishing (i.e. pre-evisceration), the Enterobacteriaceae numbers were significantly ($P < 0{\cdot}001$) decreased but increased during evisceration (Table 1).

Escherichia coli was recovered from the majority of pre-scald carcasses with the counts remaining relatively constant throughout the processing day ($1{\cdot}00 \times 10^4$ cfu 100 cm^{-2}). *Escherichia coli* numbers recovered from carcasses pre-dry polisher were on average 6-fold lower compared to pre-scald (Table 1). Evisceration caused an increase in *E. coli* counts on several carcasses but on average this was not significant ($P > 0{\cdot}10$).

Table 1 Counts of aerobes, Enterobacteriaceae and *Escherichia coli* recovered from the brisket of pork carcasses at different times and points in processing

Sampling time (h:min into activity)		Pre-scald*	Pre-dry polisher*	Pre-evisceration*	Post-evisceration*
Total aerobic count	0:10	$2{\cdot}00 \times 10^7$	$5{\cdot}01 \times 10^6$	$1{\cdot}00 \times 10^7$	$3{\cdot}16 \times 10^6$
	1:00	$1{\cdot}58 \times 10^6$	$3{\cdot}98 \times 10^7$	$1{\cdot}58 \times 10^6$	$3{\cdot}98 \times 10^6$
	1:45	$3{\cdot}16 \times 10^7$	$1{\cdot}58 \times 10^6$	$2{\cdot}51 \times 10^6$	$3{\cdot}16 \times 10^6$
	2:30	$2{\cdot}00 \times 10^7$	$3{\cdot}16 \times 10^6$	$6{\cdot}31 \times 10^6$	$2{\cdot}00 \times 10^6$
	3:15	$2{\cdot}00 \times 10^7$	$2{\cdot}51 \times 10^6$	$1{\cdot}58 \times 10^7$	$2{\cdot}00 \times 10^6$
	4:00	$1{\cdot}26 \times 10^7$	$1{\cdot}00 \times 10^6$	$1{\cdot}26 \times 10^7$	$6{\cdot}31 \times 10^6$
	5:15	$1{\cdot}26 \times 10^7$	$5{\cdot}01 \times 10^6$	$6{\cdot}31 \times 10^6$	$2{\cdot}00 \times 10^6$
	6:00	$2{\cdot}51 \times 10^7$	$7{\cdot}94 \times 10^6$	$7{\cdot}94 \times 10^6$	$1{\cdot}00 \times 10^7$
	7:00	$1{\cdot}26 \times 10^7$	$6{\cdot}31 \times 10^6$	$1{\cdot}00 \times 10^7$	$5{\cdot}01 \times 10^6$
	8:20	$1{\cdot}00 \times 10^8$	$2{\cdot}00 \times 10^6$	$1{\cdot}26 \times 10^7$	$3{\cdot}16 \times 10^6$
	Ave	$2{\cdot}56 \times 10^7$	$7{\cdot}43 \times 10^6$	$8{\cdot}57 \times 10^6$	$4{\cdot}08 \times 10^6$
Enterobacteriaceae	0:10	$3{\cdot}98 \times 10^4$	$1{\cdot}00 \times 10^4$	$3{\cdot}98 \times 10^2$	$6{\cdot}31 \times 10^3$
	1:00	ND	$6{\cdot}31 \times 10^4$	$2{\cdot}51 \times 10^3$	$6{\cdot}31 \times 10^2$
	1:45	$6{\cdot}31 \times 10^4$	$3{\cdot}98 \times 10^2$	$5{\cdot}01 \times 10^1$	$3{\cdot}98 \times 10^3$
	2:30	$6{\cdot}31 \times 10^4$	$2{\cdot}00 \times 10^3$	$1{\cdot}26 \times 10^3$	$5{\cdot}01 \times 10^2$
	3:15	$5{\cdot}01 \times 10^4$	$2{\cdot}51 \times 10^3$	$5{\cdot}01 \times 10^2$	$2{\cdot}00 \times 10^3$
	4:00	$3{\cdot}98 \times 10^4$	$1{\cdot}00 \times 10^3$	$2{\cdot}00 \times 10^3$	$7{\cdot}94 \times 10^3$
	5:15	$1{\cdot}00 \times 10^5$	$1{\cdot}58 \times 10^4$	$1{\cdot}00 \times 10^3$	$2{\cdot}00 \times 10^2$
	6:00	$6{\cdot}31 \times 10^4$	$5{\cdot}01 \times 10^3$	$1{\cdot}26 \times 10^3$	$6{\cdot}31 \times 10^3$
	7:00	$1{\cdot}26 \times 10^4$	$2{\cdot}00 \times 10^4$	$6{\cdot}31 \times 10^3$	$5{\cdot}01 \times 10^3$
	8:20	$1{\cdot}00 \times 10^7$	$1{\cdot}26 \times 10^4$	$3{\cdot}16 \times 10^3$	$1{\cdot}00 \times 10^4$
	Ave	$1{\cdot}05 \times 10^6$	$1{\cdot}32 \times 10^4$	$1{\cdot}84 \times 10^3$	$4{\cdot}29 \times 10^3$
Escherichia coli	0:10	$1{\cdot}58 \times 10^3$	$1{\cdot}00 \times 10^3$	$3{\cdot}16 \times 10^1$	$1{\cdot}58 \times 10^2$
	1:00	ND	$5{\cdot}01 \times 10^4$	$1{\cdot}58 \times 10^3$	$1{\cdot}58 \times 10^1$
	1:45	$7{\cdot}94 \times 10^4$	$5{\cdot}01 \times 10^2$	$5{\cdot}01 \times 10^1$	$3{\cdot}16 \times 10^2$
	2:30	$1{\cdot}00 \times 10^4$	$3{\cdot}98 \times 10^2$	$1{\cdot}00 \times 10^2$	$6{\cdot}31 \times 10^1$
	3:15	$2{\cdot}51 \times 10^4$	$2{\cdot}51 \times 10^3$	$7{\cdot}94 \times 10^1$	$2{\cdot}51 \times 10^3$
	4:00	$2{\cdot}51 \times 10^4$	$3{\cdot}98 \times 10^2$	$1{\cdot}26 \times 10^2$	$6{\cdot}31 \times 10^1$
	5:15	$5{\cdot}01 \times 10^4$	$3{\cdot}98 \times 10^3$	$3{\cdot}98 \times 10^2$	$7{\cdot}94 \times 10^2$
	6:00	$6{\cdot}31 \times 10^4$	$2{\cdot}51 \times 10^3$	$3{\cdot}16 \times 10^2$	$3{\cdot}16 \times 10^2$
	7:00	$3{\cdot}16 \times 10^4$	$2{\cdot}00 \times 10^3$	$1{\cdot}26 \times 10^3$	$1{\cdot}00 \times 10^3$
	8:20	$1{\cdot}00 \times 10^5$	$5{\cdot}01 \times 10^2$	$1{\cdot}00 \times 10^2$	$2{\cdot}00 \times 10^2$
	Ave	$3{\cdot}86 \times 10^4$	$6{\cdot}39 \times 10^3$	$5{\cdot}18 \times 10^2$	$5{\cdot}44 \times 10^2$

*cfu 100 cm^{-2}.
ND: not detected (< 50 cfu 100 cm^{-2}).

Bacterial counts of environmental samples

Prior to the start of processing, a relatively high total aerobic count (including Enterobacteriaceae and *E. coli*) was recovered from the scraper blades (Table 2).

Although the total aerobic counts on scraper blades remained essentially constant, the numbers of *E. coli* and Enterobacteriaceae had increased significantly ($P < 0{\cdot}01$) by the end of the processing day. The dry polisher blades were also found to harbour a substantial Enterobacteriaceae and

Table 2 Bacterial counts derived from environmental sources within the pork processing plant

Time into activity (h:min)	Scald water‡	Scraper blade§	Dry polisher§	Wet polisher§	Band-saw§	Butcher's hand§	Butcher's knife§
Total aerobic count							
0:00	$7{\cdot}94 \times 10^2$	$1{\cdot}74 \times 10^8$	$3{\cdot}72 \times 10^7$	$7{\cdot}76 \times 10^8$	$1{\cdot}48 \times 10^5$	$5{\cdot}62 \times 10^6$	NT
4:00	NT*	$1{\cdot}45 \times 10^8$	$1{\cdot}55 \times 10^8$	$5{\cdot}75 \times 10^8$	$8{\cdot}71 \times 10^7$	$2{\cdot}04 \times 10^6$	$3{\cdot}16 \times 10^8$
8:30	$3{\cdot}98 \times 10^4$	$1{\cdot}55 \times 10^8$	$4{\cdot}47 \times 10^6$	$1{\cdot}91 \times 10^8$	$2{\cdot}14 \times 10^7$	$1{\cdot}12 \times 10^6$	NT
Enterobacteriaceae							
0:00	ND†	$3{\cdot}55 \times 10^5$	$7{\cdot}08 \times 10^4$	ND	ND	ND	NT
4:00	NT	$2{\cdot}34 \times 10^5$	$2{\cdot}19 \times 10^4$	$2{\cdot}34 \times 10^5$	$1{\cdot}00 \times 10^7$	$8{\cdot}51 \times 10^4$	$3{\cdot}98 \times 10^4$
8:30	ND	$1{\cdot}70 \times 10^7$	$4{\cdot}27 \times 10^5$	$2{\cdot}29 \times 10^4$	$7{\cdot}94 \times 10^6$	$4{\cdot}07 \times 10^5$	NT
Escherichia coli							
0:00	ND	$1{\cdot}62 \times 10^5$	$3{\cdot}02 \times 10^3$	ND	ND	ND	NT
4:00	NT	$2{\cdot}19 \times 10^5$	$1{\cdot}51 \times 10^6$	$1{\cdot}26 \times 10^4$	$4{\cdot}37 \times 10^6$	$2{\cdot}34 \times 10^5$	$3{\cdot}31 \times 10^4$
8:30	ND	$4{\cdot}37 \times 10^6$	$6{\cdot}46 \times 10^4$	$7{\cdot}08 \times 10^4$	$1{\cdot}55 \times 10^5$	$8{\cdot}91 \times 10^3$	NT

*NT: not tested.
†ND: not detected.
‡cfu ml^{-1}.
§cfu per unit.

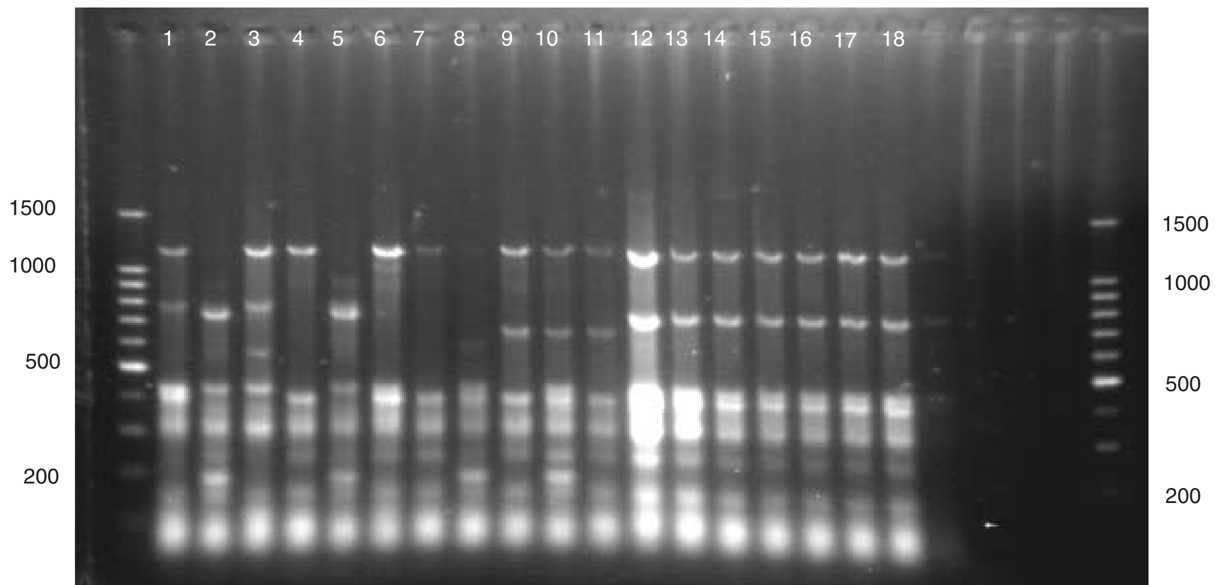

Fig. 2 Representative DNA fingerprints obtained from ERIC-PCR of *Escherichia coli* isolates derived from slaughterhouse samples. Aliquots (10 μl) of PCR products were loaded on a 2% w/v agarose gel and electrophoresed for 2·5 h at 70 V. Outer bands are 100 bp ladder. Lanes 9–18 show the DNA fingerprint pattern of genotype VI isolated from a scraper blade at the start (9–12) and mid point of activity (13–15) and from carcasses (post-scrape 16 and pre-evisceration 17–18). Also shown is a selection of other *E. coli* genotypes recovered (lanes 1–8) during sampling. Scraper blade (genotype V: lane 1), dry polisher and wet polisher (genotype X: lanes 2 and 5), dry polisher (genotype III: lane 3), pre-evisceration carcass (genotype VII: lane 4), post-evisceration carcass (genotype XI and IX: lane 6 and lane 7, respectively) and pre-scald carcass (genotype I: lane 8)

E. coli population prior to the start of activity (Table 2). In contrast no *E. coli* or Enterobacteriaceae were recovered from the blades of the wet polisher (the operation that follows singeing) prior to the start of processing. However, samples taken from the wet polisher blades four hours into the activity and at the end of processing showed an increase in counts of the indicator organisms (Table 2). A similar observation was made for the halving band-saw, butcher's hand and evisceration knife. This would suggest that although the surfaces were initially free of *E. coli* and Enterobacteriaceae at the start of processing, contamination derived from carcasses readily occurred during the activity.

The total viable count of scald water during the processing day increased, but no *E. coli* or Enterobacteriaceae were recovered. This may be expected given the temperature (69 °C) at which the scald tank was run.

Genotyping of *Escherichia coli* isolates derived from environmental samples

The fingerprint patterns generated by ERIC-PCR typically resulted in 4–7 bands ranging between 150 and 1000 bp (Fig. 2). From the DNA fingerprints of 97 isolates taken from the surface of carcasses and environmental sources 29 genotypes were identified numbered I to XXIX (Table 3).

Prior to the start of processing, the scraper blades were found to harbour a diverse range of *E. coli* genotypes

Table 3 *Escherichia coli* genotypes recovered from environmental samples and carcass briskets at different times during processing

	Genotypes		
	Start of activity	Mid-point of activity	End of activity
Environmental samples			
Scraper blade	I	V	IV
	IV	VI	
	VI	VII	
	XXVII		
Dry polisher	VI	II	III
	XI	VII	XI
		VIII	
		X	
Wet polisher	No *E. coli* recovered	III	III
		IV	VII
		IX	XXIII
		X	XXVI
Band-saw	No *E. coli* recovered	IV	III
		IX	
		X	
Butcher's hand (after	X	XXIX	II
handling initial carcasses)	XX	XXVIII	IV
	XXV		
Carcass brisket*			
Pre-scald	I	II	V
	XIII	V	X
	XIV	VII	XXIII
Post-scraper	I	V	II
	II	VI	XXII
	XI		
	XV		
	XVI		
Pre-evisceration	VI	V	II
	XVIII	VII	XVII
			XXI
Post-evisceration	I	I	II
	VI	II	XII
	IX	XXIV	XIX
	XI	XXVI	XXIX

*Carcass samples taken at the start, mid-point and end of activity

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compared to samples derived from the dry polisher. After 4 h activity the *E. coli* genotypes recovered from the scraper blades had altered, although type VI was again recovered. A greater diversity of genotypes was recovered from the dry polisher at this time and included type VII, which was also present on the scraper blade (Table 3). The same *E. coli* genotype was also recovered from a pre-scald carcass, suggesting that this strain had been transferred to the scraper and dry polisher blades during the course of processing.

Although the wet polisher was initially free of *E. coli*, a diverse range of genotypes accumulated during the initial 4 h of processing. Interestingly isolate IV, initially found on the scraper at the start of the day, was isolated on the wet polisher blade. Genotype X was present on the dry polisher blade and was also recovered from the wet polisher and band saw along with isolate IV, which was initially recovered from the scraper blade. This would suggest that as processing continued the *E. coli* present on scraper blade surfaces were subsequently distributed via carcasses throughout the line.

Upon completion of processing, genotype IV, which had initially been recovered from scraper blades prior to the start of processing, was again present. The dry polisher also appeared to have a less diverse population, with isolate III being predominant. This isolate was found on the wet polisher blade and band-saw but not on any of the carcasses. Genotype II recovered from the butcher's hand could be traced to the wet and dry polishers. The same genotype was also recovered from carcasses at different points in the process throughout the day (Table 3).

As random sampling of carcasses at each stage was applied, it was not possible to trace the changing *E. coli* genotypes present on individual carcasses at each stage of processing. Nevertheless, it was apparent that the majority of isolates present on carcasses could be traced to an environmental source. In addition, genotypes recovered from carcasses at pre-scald were subsequently isolated from environmental sources (Table 3). This would suggest that *E. coli* genotypes are frequently transferred between carcasses and contact surfaces.

DISCUSSION

By using a combination of plate counts and DNA-fingerprint studies it has been demonstrated that cross-contamination between carcasses and contact surfaces progressively increases during the pork processing activity. As the total aerobic counts obtained appeared invariant to the time or stage of processing, such data could not be used to determine sites of cross-contamination. Conversely, Enterobacteriaceae and *E. coli* levels did provide a basis for determining cross-contamination events throughout the processing line. In terms of Enterobacteriaceae numbers, the key cross-contamination site was evisceration, where a significant ($P < 0{\cdot}05$) increase in counts on carcasses was observed. This is in agreement with Rivas *et al.* (2000) where evisceration was highlighted as a key cross-contamination event in pork production.

In the present study it was not possible to sample carcasses following scalding and singeing. Although scalding and singeing are specifically intended to remove hair from the surface of pig carcasses, both have been demonstrated to remove a substantial proportion of the skin microflora (Sorquist and Danielssen 1986; Borch *et al.* 1996). However, scraping and polishing have been reported to re-contaminate carcasses (Gill and Bryant 1993; Rivas *et al.* 2000; Yu and Palumbo 2000). Therefore, it is probable that within the slaughter line sampled, additional cross-contamination sites were present. This was demonstrated through DNA fingerprinting, with *E. coli* isolates present on scraper/dry polisher blades prior to the start of processing being subsequently isolated from the wet polisher. Giovannacci *et al.* (2001) reported that the greater diversity of *Salmonella* genotypes recovered from pig carcasses between de-hairing and splitting was as a result of extensive cross-contamination between carcasses. The relatively high level of wet scraper blade contamination at an early period of processing may be unexpected considering that the carcasses would have been subjected to singeing where the surface temperature would reach in excess of 100 °C. However, it has been shown that although singeing reduces counts by *ca* 2 log units it is insufficient to fully decontaminate carcasses (Gill and Bryant 1992, 1993). Therefore, it is likely that the residual *E. coli* on singed carcasses was transferred to the wet polisher blades and subsequently to other carcasses, a process augmented by the re-circulating water used in this process. By the end of processing, *E. coli* genotypes originally present on the scraper and polisher blades had transferred to the band-saw in addition to the butcher's hands. This clearly illustrates that although good manufacturing practices are employed and segregation of dirty/clean areas was applied, the transfer of *E. coli* (and presumably other pathogens) still occurred.

From the range of genotypes identified, it was apparent that *E. coli* types VI and X persisted within the processing environment compared to more transient types recovered. Persistent *Listeria monocytogenes* pulsotypes can become established within slaughterhouse environments whilst others were more transient in nature (Giovannacci *et al.* 1999; Senczek *et al.* 2000). The reasons for the persistence of certain strains over others remain unclear but it is likely to be attributed to the inherent resistance of the cell that adapts to the processing environment. This has been observed previously for endemic *Staphyloccus aureus* isolated from poultry processing plants that have enhanced resistance to

sodium hypochlorite disinfectant used during hygiene operations (Bolton *et al.* 1988).

Increased surface contamination during evisceration is well documented (Gill and Jones 1997) where bacteria present within the intestinal tract, in addition to mouths/tonsils (Gill and Jones 1998; Autio *et al.* 2000) can contaminate the carcass. It was interesting to note that the *E. coli*/Enterobacteriaceae loading on post-eviscerated carcasses was relatively high despite the carcass being washed via a pressure hose. However, it has previously been reported that carcass washing primarily re-distributes bacteria across the surface as opposed to removing contamination (Dickson and Anderson 1992; Rivas *et al.* 2000). Therefore, the high variation in counts recovered from post-eviscerated carcasses was not only due to the efficiency by which the process was performed but also as a result of re-distribution of bacteria due to the water wash. The finding that a number of genotypes could be traced to environmental sources as opposed to the emergence of new types (originating from the GI tract) supports this view. Therefore, the evisceration process may not contribute to carcass cross-contamination as initially envisaged.

Although indicator bacterial counts are useful for monitoring the standard of plant hygiene, their application in identifying cross-contamination sites within slaughter lines is limited. Counts of indicator organisms would demonstrate gross cross-contamination events throughout the processing line but not the subtle flora changes as revealed by applying molecular typing. Consequently the only control point in the current process is the scalding operation. Potential steps for controlling cross-contamination within the slaughter line are difficult to envisage considering the intensive nature of modern day lines. By using the level of contamination of carcasses at pre-evisceration it can be assumed that the wet polisher blades were contaminated early into the processing activity. Whether this would have been delayed by ensuring that scraper and dry polisher blades were adequately decontaminated prior to processing cannot be stated. Nevertheless, in-place cleaning regimes applied during processing may minimize the frequency of cross-contamination. An alternative measure would be to introduce a pre-evisceration intervention step such as steam pasteurization, hot water wash or organic acid sprays. Preliminary studies have shown potential (Gill *et al.* 1998) and appear to be the most practical approach in controlling pathogen contamination on pork carcasses, although the detrimental impact on the visual appearance of meat surfaces could be a limitation.

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