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Enterococcus in retail premises

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Executive Summary

Recent investigations into the microbiological quality of the red meat production system identified that the microbiological indicators of hygiene and imminent spoilage were higher at the retail level than on carcasses and trim at abattoirs. Of particular note was an increase in antimicrobial resistant (AMR) *Enterococcus* isolates and an associated decrease in the variability of *Enterococcus* species present at the retail level. The reasons for the decrease in species variation or the increase in AMR from abattoir carcases to retail meat are unknown. In order to determine if aspects of the retail chain such as retail staff, processing equipment or the meat itself contribute to these findings a study was conducted to evaluate the genotypic relatedness of *Enterococcus* isolated from a retail butchery during one day of retail trade.

A total of 180 samples comprising 50 product and 130 environmental samples were collected during a single trading day at a large retail butchery. *Enterocccus* sp. were isolated from 75 (41.7%) samples collected with 33 (66%) product samples and 42 (32.3%) environmental samples yielding isolates. Thirty-four of the 75 positive samples had quantifiable numbers of *Enterococcus* sp.. Product samples generated 10 of the 34 quantifiable numbers with counts ranging from 1.00 to 2.58 $\log_{10}$CFU/cm or g. The counts of *Enterococcus* sp. in environmental samples were, on average, higher than product samples and ranged from 1.30 to 4.67 $\log_{10}$CFU/cm. Counts from hands and knives were generally higher and occurred at greater frequency than surface samples. *Enterococcus faecalis* was most frequently recovered; accounting for almost 60% of isolates. *E. faecium* (14.6%) and *E. gallinarum* (12.6%) comprised the majority of remaining isolates with *E. hirae* (8.7%) and *E. durans* (4.9%) present at lower levels. Of the 20 isolates from 16 positive beef carcase or beef mince samples 95% were *E. faecalis*. In contrast, of the 23 isolates from 17 positive lamb carcase or diced lamb samples only 21.7% were *E. faecalis*. Interestingly, *E. faecalis* were isolated from diced lamb samples but not from lamb carcases despite all remaining *Enterococcus* species identified in this study being present on lamb carcases.

Examination of 57 *E. faecalis* and 10 *E. faecium* isolates using *Sma*I-PFGE defined 34 *E. faecalis* and 4 *E. faecium* PFGE types at 100% similarity and 20 *E. faecalis* and 4 *E. faecium* PFGE types at 90% similarity. The relatively low number of *E. faecium* isolates examined meant that detection of cross-contamination events was difficult with only one *Sma*I-PFGE type comprising more than 2 samples. Similar heterogeneity was observed with *E. faecalis* isolates with just eleven (32%) of *Sma*I-PFGE types comprised of isolates from more than one sample. The isolate source data from related isolates indicate that they were recovered from a variety of

different raw meat, retail premise surfaces and environmental sources. Such data generally provide evidence for cross contamination and distribution throughout the retail premises. Indeed *E. faecalis* isolates from diced lamb were shown to be identical to those found on beef carcases. In the absence of similar *E. faecalis* isolates being present on the lamb carcases tested it is likely that cross-contamination has occurred in this instance. However, whilst there is some evidence for potential cross-contamination occurring within the butchery, the diversity of *Enterococcus* isolates from this study indicated that the majority of contamination observed is transient in nature. Although the results of this study do not permit for the direction of cross-contamination to be conclusively determined, chronological evaluation of processes and procedures within the butchery suggest that both incoming raw meat and environmental factors such as the hands of staff play a role in the introduction and transfer of *Enterococcus*. Repeat sampling at the same butchery could assist in identifying cross-contamination events that occur on a regular basis and the direction in which they occur. It would also allow the identification of clones that persist within the butchery over time even at low prevalences.

Background

The recent microbiological survey of retail product project indicated that although microbiological indicators of hygiene and imminent spoilage on carcases and trim were only present at low frequencies and concentration, at a retail level, levels were higher than expected. Isolates from this project were analysed for antimicrobial resistance (AMR) by FSA as part of the project looking at AMR in red meat - both on carcases and at retail outlets (A.MFS.0061). *Enterococcus faecium* and in particular *Enterococcus faecalis* are more prevalent in retail meat than other *Enterococcus* species found on abattoir carcases. Furthermore, *Enterococcus* isolates from retail meat have been shown to have increased AMR prevalences with respect to abattoir isolates. The reasons for the decrease in species variation or the increase in AMR from abattoir carcases to retail meat are unknown. In addition, the role that individual components of the retail chain such as retail staff, processing equipment or the meat itself play in the transfer of contamination or AMR between products and over time is unknown. In order to investigate this a large retail butchery will be intensively sampled for *Enterococcus* isolates throughout one day of retail trade to identify sources of contamination. The genotypic relatedness of *Enterococcus* isolates will be examined using Pulsed Field Gel Electrophoresis (PFGE) to determine whether contamination of retail product originates from raw meat when it enters the premise, whether there are environmental reservoirs of contamination within the premise, or whether contamination of meat comes from people working with the product. The AMR phenotypes of all isolates will be determined and subsequently combined with PFGE data to establish if the increased AMR prevalences observed in previous studies is related to any specific retail process.

Methods

Sampling

Sample collection occurred in 2 phases. Phase 1 involved the collection of baseline samples prior to the commencement of retail trade. Phase 2 involved the collection of samples during retail trade operations. Both phases were completed on the same day with the initial baseline phase conducted prior to the start of retail business and the retail phase being conducted during business hours. The samples collected in each phase are outlined below.

Phase 1: Initial baseline phase

- 20 environmental samples (staff hands, work surfaces, knives etc) – 10 of which were selected for re-sampling during phase 2
- 15 beef samples – carcass swabs, samples of incoming and existing product
- 15 lamb samples – carcass swabs, samples of incoming and existing product

Phase 2: Retail phase

- 10 environmental samples selected during Phase 1 were re-tested at regular intervals throughout the day
- A total of 10 beef mince samples were collected at regular intervals throughout the day.
- A total of 10 diced lamb samples were collected at regular intervals throughout the day
- 20 environmental samples (same locations as phase 1) were collected at the end of the retail phase immediately following the final clean up.

A total of 180 samples were collected comprising 50 product samples and 130 environmental samples. With the exception of the beef mince and diced lamb samples where a 100g portion was collected, all samples represent the swabbing of 100 cm^2 using Whirlpak sponges (Nasco) rehydrated in 20 ml of buffered peptone water (BPW; Oxoid). Initial suspensions were prepared by adding 80 ml BPW to each 100cm^2 swab or by mixing a 25g portion of product with 225 ml BPW. All samples were stomached for 1 min prior to further processing.

Temperature logging

The temperatures of an open chest meat display cabinet and the ambient temperature of the retail butchery were recorded. Readings were collected at 5 minute intervals using Tinyview Plus data loggers (Gemini Data Loggers).

Detection and enumeration of *Enterococcus* isolates

Enterococcus quantitation

- Serial 10-fold dilutions of the initial suspensions were prepared and spread plated onto Slanetz-Bartley agar (Schenker, Melbourne) and incubated at 35℃ for 48h ±2 hours.
- Presumptive *Enterococcus* counts were determined by counting pink or dark red colonies, with a narrow whitish border.
- Up to 10 presumptive *Enterococcus* isolates were plated onto nutrient agar and incubated for 18h ±2 at 37℃
- Presumptive *Enterococcus* isolates were speciated using PCR or the VITEK 60 system.
- Up to 2 *Enterococcus* isolates per samples were stored at -80℃ using pro tect beads

Enterococcus detection

- One ml of the initial suspension were transferred to 9 ml Enterococcosel broth (BD, USA) and incubated o/n at 37℃. The resulting enrichment s were plated onto Slanetz-Bartley agar and incubated at 35℃ for 48 hours.
- Presumptive *Enterococcus* isolates were determined by counting pink or dark red colonies, with a narrow whitish border and up to 10 presumptive *Enterococcus* isolates were plated onto nutrient agar and incubated for 18h ±2 at 37℃
- Presumptive *Enterococcus* isolates were speciated using PCR or the VITEK 60 system.
- Up to 2 *Enterococcus* isolates per samples were stored at -80℃ using pro tect beads

Molecular typing of Enterococcus faecalis isolates

Pulsed field gel electrophoresis (PFGE) was used to determine the relationship between *Enterococcus* isolates following the method of Dahl et al. (1999) using the restriction enzyme *Sma*I (referred to here as *Sma*I-PFGE). Electrophoresis was performed using a CHEF-DRIII apparatus (BioRad) in 1% PFGE certified agarose (BioRad). Gels were stained with ethidium bromide, visualised under UV and images stored using Gene Genius (Syngene). Phenograms of *Enterococcus* isolates were generated using GelCompar II (Applied Maths) with Dice correlation and UPGMA clustering with a position tolerance of 1.8%, increasing by 0.5% and optimization of 1%.

AMR testing

The resistance phenotype of up to two *Enterococcus* isolates per sample were determined using the VITEK 60 system with the GPS-109 card. The GPS-109 card includes the

antimicrobials ampicillin, gentamicin-500, levofloxacin, linezolid, penicillin-G, quinupristin / dalfopristin, streptomycin-2000, tetracycline and vancomycin.

Results and discussion

A total of 180 samples comprising 50 product and 130 environmental samples were collected during a single trading day at a large retail butchery. The temperatures recorded during the sampling phases are shown in Figure 1. The results of testing are shown in Table 1. *Enterocccus* sp. were isolated from 75 (41.7%) samples collected with 33 (66%) product samples and 42 (32.3%) environmental samples yielding isolates. Thirty-four of the 75 positive samples had quantifiable numbers of *Enterococcus* sp.. Product samples generated 10 of the 34 quantifiable numbers with counts ranging from 1.00 to 2.58 $\log_{10}$CFU/cm or g. The counts of *Enterococcus* sp. in environmental samples were, on average, higher than product samples and ranged from 1.30 to 4.67 $\log_{10}$CFU/cm. Counts from hands and knives were generally higher and occurred at greater frequency than surface samples. Furthermore *Enterococcus* isolates were obtained from a knife or hand samples in eight of the nine phase 2 sampling periods.

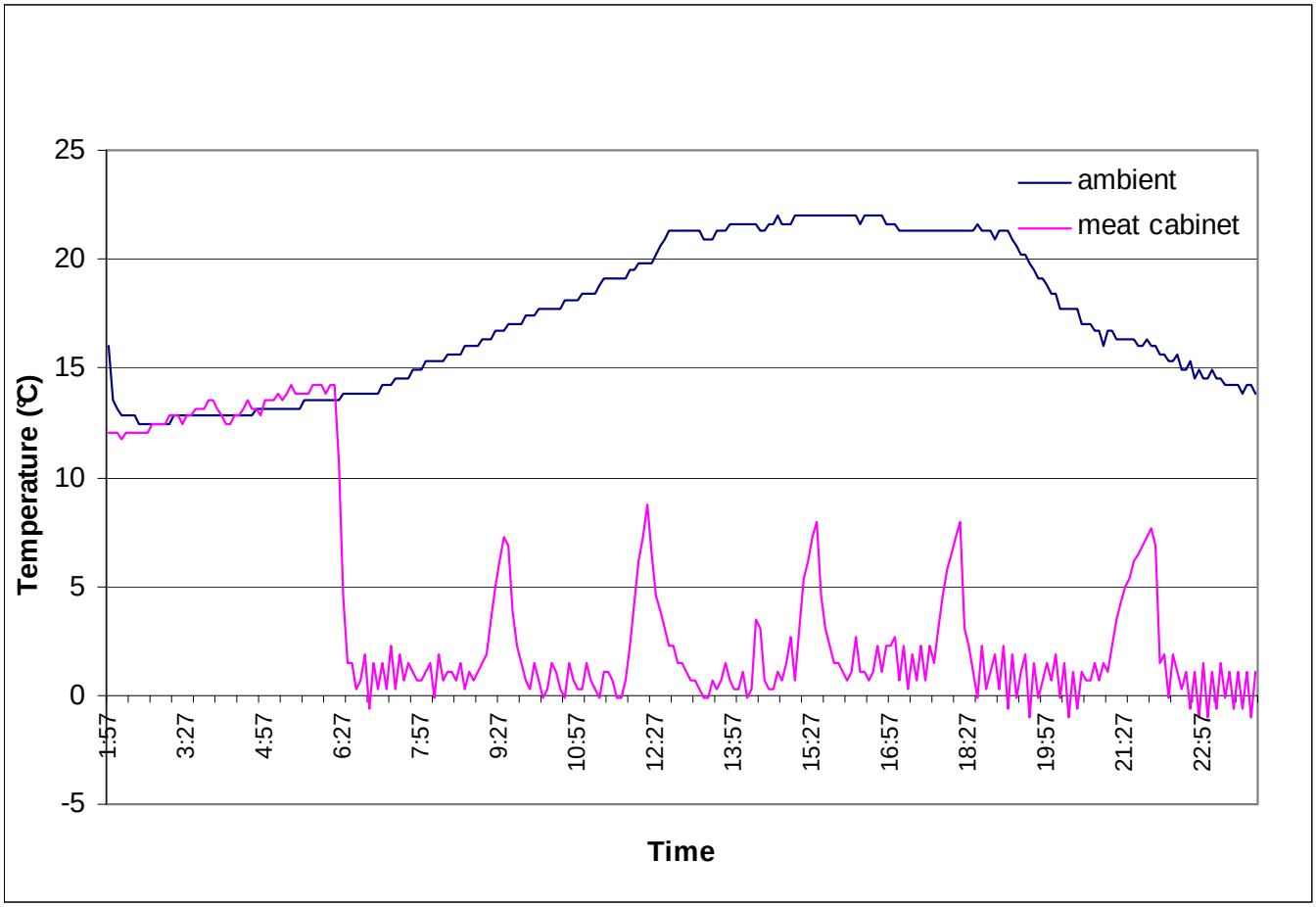

Figure 1. Chest meat display cabinet and ambient temperatures logged during sample collection phases

A total of 103 isolates were recovered from 75 positive samples. Of the samples that yielded two isolates only seven had more than one species present. *Enterococcus faecalis* was most frequently recovered; accounting for almost 60% of isolates. Similar high prevalences of *E. faecalis* were also observed in the 2004/2005 Microbiological Survey of Australian Red Meat (retail) and from in-line sampling of butchers premises (A.MFS.0061). *E. faecium* (14.6%) and *E. gallinarum* (12.6%) comprised the majority of remaining isolates with *E. hirae* (8.7%) and *E. durans* (4.9%) present at lower levels. Of the 20 isolates from 16 positive beef carcase or beef mince samples 95% were *E. faecalis*. In contrast, of the 23 isolates from 17 positive lamb carcase or diced lamb samples only 21.7% were *E. faecalis*. Interestingly, *E. faecalis* were isolated from diced lamb samples but not from lamb carcases despite all remaining *Enterococcus* species identified in this study being present on lamb carcases. No relationships between species and the type of environmental sample could be found with *E. faecalis, E. gallinarum* and *E. faecium* present in similar proportions in these samples. AMR testing of all *Enterococcus* isolates determined that the overall level of antimicrobial resistance was generally low with 86 (83.5%) isolates susceptible to all antimicrobials tested. Multiple antimicrobial resistance was observed in just seven (6.8%) isolates with resistance to streptomycin-2000 and tetracycline the most commonly observed resistance pattern accounting for 35.3% of all resistant isolates. Vancomycin resistance was observed in four (3.9%) isolates recovered from beef carcase, knife and bandsaw samples. The levels of AMR in *Enterococcus* isolates in this study, particularly for gentamicin, are lower than those observed in previous A.MFS.0061 related projects and consequently does not enable conclusions relating to the transfer of AMR *Enterococcus* within the retail butchery to be made.

Table 1. Detection, quantitation, identification and AMR phentotypes of *Enterococcus* isolates from a retail butchery

Sample	Sample type	Sample Time	Quantitation procedure			Enrichment procedure		
			Quantitation[a]	Species	AMR phenotype	Enrichment	Species	AMR phenotype
A 1	Beef carcase - boning room	2:50 AM						
A 2	Beef carcase - boning room	2:50 AM						
A 3	Beef carcase - boning room	2:50 AM						
A 4	Beef carcase - boning room	2:50 AM						
A 5	Beef carcase - boning room	2:50 AM						
A 6	Beef carcase - boning room	2:50 AM				✓	*faecalis*	ND
A 7	Beef carcase - boning room	2:50 AM				✓	*faecalis*	ND
A 8	Beef carcase - retail area	8:00 AM						
A 9	Beef carcase - retail area	8:00 AM						
A 10	Beef carcase - retail area	8:00 AM						
A 11	Beef carcase - retail area	8:00 AM				✓	*faecalis*	ND
A 12	Beef carcase - retail area	8:00 AM				✓	*faecalis*	ND
A 13	Beef carcase - retail area	8:00 AM				✓	*faecalis*	ND
A 14	Beef carcase - retail area	8:00 AM	2.28	*faecalis*	ND	✓	*faecalis*	ND
A 15	Beef carcase - retail area	8:00 AM	2.04	*faecalis*	ND	✓	*faecalis*	van
A 16	Lamb carcase - boning room	2:50 AM				✓	*hirae*	ND
A 17	Lamb carcase - boning room	2:50 AM				✓	*faecium*	ND
A 18	Lamb carcase - boning room	2:50 AM						
A 19	Lamb carcase - boning room	2:50 AM				✓	*gallinarum*	str-2000, tet
A 20	Lamb carcase - boning room	2:50 AM				✓	*hirae*	ND
A 21	Lamb carcase - boning room	2:50 AM						
A 22	Lamb carcase - boning room	2:50 AM	1.85	*faecium*	amp, pen-G, str-2000, tet	✓	*gallinarum*	ND
A 23	Lamb carcase - retail area	8:00 AM	2.28	*faecium*	ND	✓	*faecium*	ND
A 24	Lamb carcase - retail area	8:00 AM	1.60	*faecium*	ND	✓	*gallinarum*	ND
A 25	Lamb carcase - retail area	8:00 AM				✓	*durans*	ND
A 26	Lamb carcase - retail area	8:00 AM	2.11	*faecium*	ND	✓	*hirae*	ND
A 27	Lamb carcase - retail area	8:00 AM	2.46	*hirae*	ND	✓	*hirae*	ND
A 28	Lamb carcase - retail area	8:00 AM				✓	*hirae*	ND
A 29	Lamb carcase - retail area	8:00 AM				✓	*durans*	ND
A 30	Lamb carcase - retail area	8:00 AM						
B 1	Beef mince - retail	7:30 AM				✓	*faecalis*	ND
B 2	Beef mince - retail	7:30 AM				✓	*faecalis*	ND
B 3	Beef mince - retail	9:45 AM						
B 4	Beef mince - retail	11:15 AM				✓	*faecalis*	ND
B 5	Beef mince - retail	1:20 PM				✓	*faecalis*	ND
B 6	Beef mince - retail	2:20 PM				✓	*faecalis*	ND

Sample	Sample type	Sample Time	Quantitation procedure			Enrichment procedure		
			Quantitation[a]	Species	AMR phenotype	Enrichment	Species	AMR phenotype
B 7	Beef mince - retail	3:18 PM				✓	*faecalis*	ND
B 8	Beef mince - retail	4:28 PM	2.58	*faecalis*	ND	✓	*faecalis*	ND
B 9	Beef mince - retail	5:20 PM				✓	*faecalis*	ND
B 10	Beef mince - retail	6:19 PM	1.30	*gallinarum*	ND	✓	*faecalis*	ND
L 1	Diced lamb - retail	9:45 AM						
L 2	Diced lamb - retail	11:15 AM						
L 3	Diced lamb - retail	12:15 PM				✓	*faecalis*	ND
L 4	Diced lamb - retail	1:20 PM				✓	*faecalis*	ND
L 5	Diced lamb - retail	2:20 PM				✓	*faecalis*	ND
L 6	Diced lamb - retail	2:20 PM				✓	*hirae*	ND
L 7	Diced lamb - retail	3:18 PM						
L 8	Diced lamb - retail	4:28 PM						
L 9	Diced lamb - retail	5:20 PM	1.00	*faecalis*	ND	✓	*faecalis*	ND
L 10	Diced lamb - retail	6:19 PM						
1	Surface - bandsaw	1:20 AM						
2	Surface - bench	1:20 AM						
3	Surface - display shelves	1:20 AM						
4	Surface - cutting board	1:20 AM						
5	Surface - chiller door	1:20 AM				✓	*hirae*	ND
6	Hands	1:20 AM						
7	Hands	1:20 AM						
8	Hands	1:20 AM						
9	Hands	1:20 AM	3.11	*faecalis*	ND	✓	*faecalis*	ND
10	Hands	1:20 AM						
11	Knife	1:20 AM						
12	Knife	1:20 AM						
13	Square bin trolley	1:20 AM						
14	Knife	1:20 AM	3.69	*faecalis*	ND	✓	*faecalis*	ND
15	Knife	1:20 AM				✓	*faecalis*	ND
16	Surface - shop drain	1:20 AM						
17	Surface - cabinet edge	1:20 AM						
18	Surface - retail prep door	1:20 AM						
19	Surface - boning hooks	1:20 AM						
20	Surface - scales	1:20 AM	2.46	*faecium*	ND			
21	Hands	4:00 AM						
22	Hands	4:00 AM	2.51	*faecalis*	str-2000, tet	✓	*faecalis*	str-2000, tet
23	Surface - bandsaw	4:00 AM				✓	*gallinarum*	lev

Sample	Sample type	Sample Time	Quantitation procedure			Enrichment procedure		
			Quantitation[a]	Species	AMR phenotype	Enrichment	Species	AMR phenotype
24	Surface - bench	4:00 AM						
25	Surface - display shelves	4:00 AM						
26	Knife	4:00 AM						
27	Knife	4:00 AM	2.98	*gallinarum*	ND	✓	*gallinarum*	ND
28	Surface - shop drain	4:00 AM						
29	Surface - cabinet edge	4:00 AM						
30	Surface - retail prep door	4:00 AM						
31	Hands	6:00 AM				✓	*faecalis*	ND
32	Hands	6:00 AM						
33	Surface - bandsaw	6:00 AM						
34	Surface - bench	6:00 AM						
35	Surface - display shelves	6:00 AM						
36	Knife	6:00 AM	3.69	*gallinarum*		✓	*gallinarum*	ND
37	Knife	6:00 AM						
38	Surface - shop drain	6:00 AM						
39	Surface - cabinet edge	6:00 AM						
40	Surface - retail prep door	6:00 AM						
41	Hands	7:30 AM				✓	*durans*	ND
42	Hands	7:30 AM	1.30	*gallinarum*	lev	✓	*gallinarum*	lev
43	Surface - bandsaw	7:30 AM	2.52	*faecium*	ND	✓	*faecium*	ND
44	Surface - bench	7:30 AM				✓	*durans*	tet
45	Surface - display shelves	7:30 AM						
46	Knife	7:30 AM						
47	Knife	7:30 AM	4.67	*gallinarum*	ND	✓	*faecalis*	str-2000
48	Surface - shop drain	7:30 AM						
49	Surface - cabinet edge	7:30 AM						
50	Surface - retail prep door	7:30 AM						
51	Hands	9:45 AM				✓	*hirae*	ND
52	Hands	9:45 AM				✓	*faecalis*	ND
53	Surface - bandsaw	9:45 AM						
54	Surface - bench	9:45 AM				✓	*faecalis*	str-2000, tet
55	Surface - display shelves	9:45 AM				✓	*faecalis*	str-2000
56	Knife	9:45 AM						
57	Knife	9:45 AM	2.32	*faecium*	ND			
58	Surface - shop drain	9:45 AM						
59	Surface - cabinet edge	9:45 AM						
60	Surface - retail prep door	9:45 AM	3.30	*faecalis*	ND			

Sample	Sample type	Sample Time	Quantitation procedure			Enrichment procedure		
			Quantitation[a]	Species	AMR phenotype	Enrichment	Species	AMR phenotype
61	Hands	11:00 AM	2.43	*faecalis*	ND	✓	*faecalis*	ND
62	Hands	11:00 AM	2.28	*faecalis*	ND	✓	*faecalis*	ND
63	Surface - bandsaw	11:00 AM						
64	Surface - bench	11:00 AM	2.23	*faecalis*	ND	✓	*faecalis*	ND
65	Surface - display shelves	11:00 AM				✓	*durans*	ND
66	Knife	11:00 AM	3.89	*faecalis*	van	✓	*faecalis*	ND
67	Knife	11:00 AM	1.30	*faecalis*	van	✓	*faecalis*	ND
68	Surface - shop drain	11:00 AM	1.48	*faecium*	ND			
69	Surface - cabinet edge	11:00 AM	2.26	*faecalis*	ND			
70	Surface - retail prep door	11:00 AM	3.01	*faecalis*	tet	✓	*faecalis*	tet
71	Hands	1:15 PM				✓	*faecalis*	ND
72	Hands	1:15 PM						
73	Surface - bandsaw	1:15 PM				✓	*faecium*	ND
74	Surface - bench	1:15 PM				✓	*faecium*	ND
75	Surface - display shelves	1:15 PM						
76	Knife	1:15 PM						
77	Knife	1:15 PM						
78	Surface - shop drain	1:15 PM	1.78	*gallinarum*	qda	✓	*faecalis*	ND
79	Surface - cabinet edge	1:15 PM						
80	Surface - retail prep door	1:15 PM	2.62	*faecalis*	str-2000, tet	✓	*faecalis*	str-2000, tet
81	Hands	2:50 PM						
82	Hands	2:50 PM	2.18	*faecalis*	ND			
83	Surface - bandsaw	2:50 PM				✓	*faecium*	van
84	Surface - bench	2:50 PM						
85	Surface - display shelves	2:50 PM						
86	Knife	2:50 PM						
87	Knife	2:50 PM						
88	Surface - shop drain	2:50 PM						
89	Surface - cabinet edge	2:50 PM						
90	Surface - retail prep door	2:50 PM						
91	Hands	4:45 PM						
92	Hands	4:45 PM						
93	Surface - bandsaw	4:45 PM						
94	Surface - bench	4:45 PM						
95	Surface - display shelves	4:45 PM						
96	Knife	4:45 PM						
97	Knife	4:45 PM						

Sample	Sample type	Sample Time	Quantitation procedure			Enrichment procedure		
			Quantitation[a]	Species	AMR phenotype	Enrichment	Species	AMR phenotype
98	Surface - shop drain	4:45 PM						
99	Surface - cabinet edge	4:45 PM						
100	Surface - retail prep door	4:45 PM						
101	Hands	6:45 PM				✓	*faecalis*	ND
102	Hands	6:45 PM						
103	Surface - bandsaw	6:45 PM	2.51	*faecium*	ND	✓	*faecalis*	ND
104	Surface - bench	6:45 PM				✓	*faecalis*	ND
105	Surface - display shelves	6:45 PM						
106	Knife	6:45 PM						
107	Knife	6:45 PM						
108	Surface - shop drain	6:45 PM						
109	Surface - cabinet edge	6:45 PM						
110	Surface - retail prep door	6:45 PM						
111	Hands	Post - clean up				✓	*faecalis*	ND
112	Hands	Post - clean up						
113	Hands	Post - clean up						
114	Hands	Post - clean up						
115	Hands	Post - clean up						
116	Surface - bandsaw	Post - clean up						
117	Surface - bench	Post - clean up						
118	Surface - display shelves	Post - clean up						
119	Surface - cutting board	Post - clean up						
120	Surface - chiller door	Post - clean up						
121	Knife	Post - clean up						
122	Knife	Post - clean up						
123	Knife	Post - clean up	2.20	*faecalis*	ND	✓	*faecalis*	ND
124	Knife	Post - clean up						
125	Knife	Post - clean up						
126	Surface - shop drain	Post - clean up						
127	Surface - cabinet edge	Post - clean up						
128	Surface - retail prep door	Post - clean up						
129	Surface - boning hooks	Post - clean up						
130	Surface - scales	Post - clean up						

[a] Counts expressed as $\log_{10}$CFU/cm^2 or g

Examination of 57 *E. faecalis* and 10 *E. faecium* isolates using *Sma*I-PFGE defined 34 and 4 unique PFGE types respectively at 100% similarity (Figures 2 & 3). When the similarity was reduced to 90% the *Sma*I-PFGE defined 20 *E. faecalis* and 4 *E. faecium* PFGE types. This is perhaps not a surprising result as genome sequencing studies have demonstrated that up to one-quarter of the *Enterococcus* genome can be made up of mobile or 'foreign' DNA including IS elements, prophage, transposons and pathogenicity islands (2, 3) The low prevalence of *E. faecium* in this study meant that the detection of cross-contamination events was difficult. Of the four *Sma*I-PFGE type groups defined, only one group comprised more than two samples. This group had similar isolates from a lamb carcase, bandsaw and a bench and although the transfer of this clone from the lamb carcase onto the bandsaw and nearby bench seems a logical progression, the lamb carcase was butchered in the boning room and would not have been in the proximity of the bandsaw or bench at any point. Therefore, if a cross-contamination event occurred it has done so via an unknown path.

Of the 34 *E. faecalis Sma*I-PFGE type groups defined, eleven (32%) were comprised of isolates from more than one sample. The isolate source data from related isolates indicate that they were recovered from a variety of different raw meat, retail premise surfaces and environmental sources (Table 2). Such data generally provide evidence for cross contamination and distribution throughout the retail premises. However, the small numbers of related isolates limit the analysis that can occur. Nevertheless there are some interesting observations to be noted. Interestingly, related isolates originating in environmental samples appeared more likely to be subsequently present in another environmental or product sample than isolates originating from the product itself. Isolates originating on hands, knives or surfaces subsequently appeared on other surfaces or in product whereas, with the exception of the type 1 isolates, the transfer of related isolates from beef product to any other sample was not observed. The type 1 isolates suggest that a cross-contamination event has occurred between a beef carcase and diced lamb product with hands or a knife a likely transfer vehicle. However, this study only identified one occasion where an isolate found on a hand or knife was subsequently found on product (Type 9 isolates). Furthermore, on the occasion that a potential link between hands and product was observed, there is circumstantial evidence to suggest that direct transfer from hands to the product did not occur on the day of sampling. The confidence to dismiss the suggested link stems from the observation that the beef carcases in the retail area are kept in a locked chiller that was unlocked immediately prior to sampling the carcases within. In addition, sample 9 (hands) was collected from an employee working in the boning room section of the retail store. The physical separation of the boning room from the retail area of the store further inhibited any possible transfer between the employee's hands and the carcase. As previously mentioned and as the above example

highlights, the low numbers of related isolates make defining relationships difficult. More definitive conclusions about the effect of hands or knives on the contamination of product would only be possible with repeated, more intensive sampling and testing.

The presence of four beef mince isolates from separate beef mince samples in type group 16 may suggest that a clone of *E. faecalis* is able to persist within the mincing equipment and consequently repeatedly contaminate batches of beef mince. Whilst further investigation of the mincing process appears warranted the possibility of the presence of a persistent clone associated with the mincing equipment is tempered somewhat by the presence of four additional type groups (18, 25, 27 and 28) in beef mince samples collected at different sampling times.

Table 2. Isolate source data for *Sma*I-PFGE related isolates

Type	Sample	Source	Time
1	A15-S	Beef Carcase - retail area	08:00
	L5-S	Diced Lamb - retail	14:20
4	A7-S	Beef Carcase - boning room	02:50
	A12-S	Beef Carcase - retail area	08:00
9	9-Q&S	Hands	01:20
	A15-Q	Beef Carcase - retail area	08:00
16	B2-S	Beef mince - retail	07:30
	B6-S	Beef mince - retail	14:20
	B8-S	Beef mince - retail	16:28
	B9-S	Beef mince - retail	17:20
24	69-Q	Surface - cabinet edge	11:00
	82-Q	Hands	14:50
25	A13-S	Beef Carcase - retail area	08:00
	B5-S	Beef mince - retail	13:20
27	B7-S	Beef mince - retail	15:18
	B10-S	Beef mince - retail	18:19
30	78-S	Surface - Shop drain	11:00
	L4-S	Diced Lamb - retail	13:20
32	71-S	Hands	13:15
	101-S	Hands	18:45
33	47-S	Knife	07:30
	54-S	Surface - bench	09:45
	55-S	Surface - display shelves	09:45
34	52-S	Hands	09:45
	62-Q&S	Hands	11:00
	64-Q&S	Surface - bench	11:00
	66-Q&S	Knife	11:00

Many *Enterococcus* types appear to enter the meat processing premises and whilst there is some evidence for potential cross-contamination occurring within the butchery, the diversity of *Enterococcus* isolates from this study indicates that the majority of contamination is transient in nature. Although the results of this study do not permit for the direction of cross-contamination to

be conclusively determined, chronological evaluation of processes and procedures within the butchery suggest that both incoming raw meat and environmental factors such as the hands of staff play a role in the introduction and transfer of *Enterococcus*. Extensive longitudinal sampling and analysis at the same butchery could assist in identifying cross-contamination events that occur on a regular basis and the direction in which they occur. It would also allow the identification of clones that persist within the butchery over time even at low prevalences.

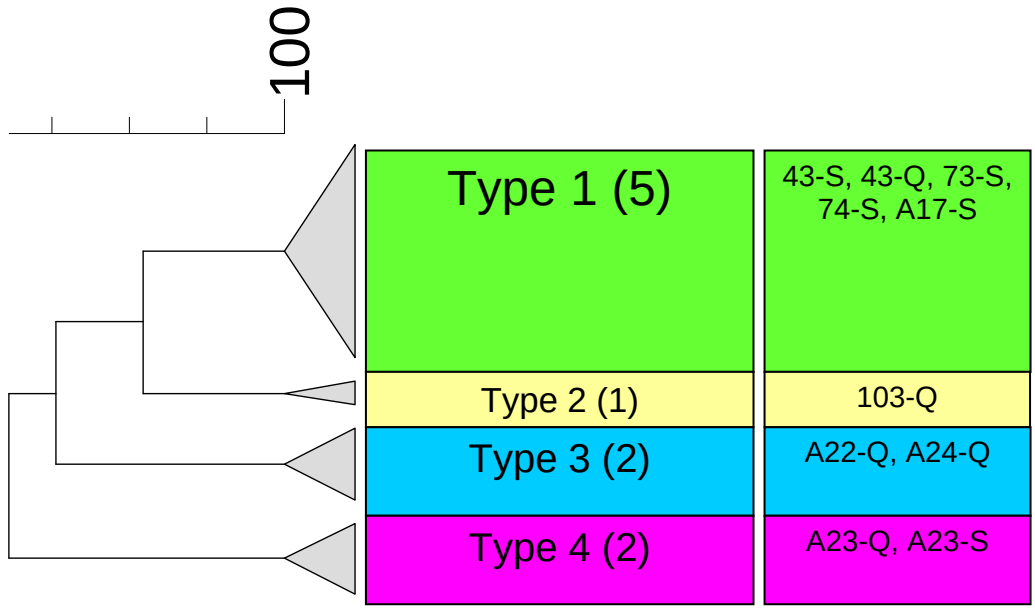

Figure 2. Phenogram of *E. faecium* isolates showing *Sma*I-PFGE types. Numbers in brackets are the number of isolates in each *Sma*I -PFGE type. Isolates included in each type are indicated. Isolates are designated ‘Q’ or ‘S’ depending on the procedure used to isolate them. ‘Q’ isolates were refer to those recovered from samples using the quantitation procedure where as isolates designated ‘S” refer to those recovered from the enrichment procedure.

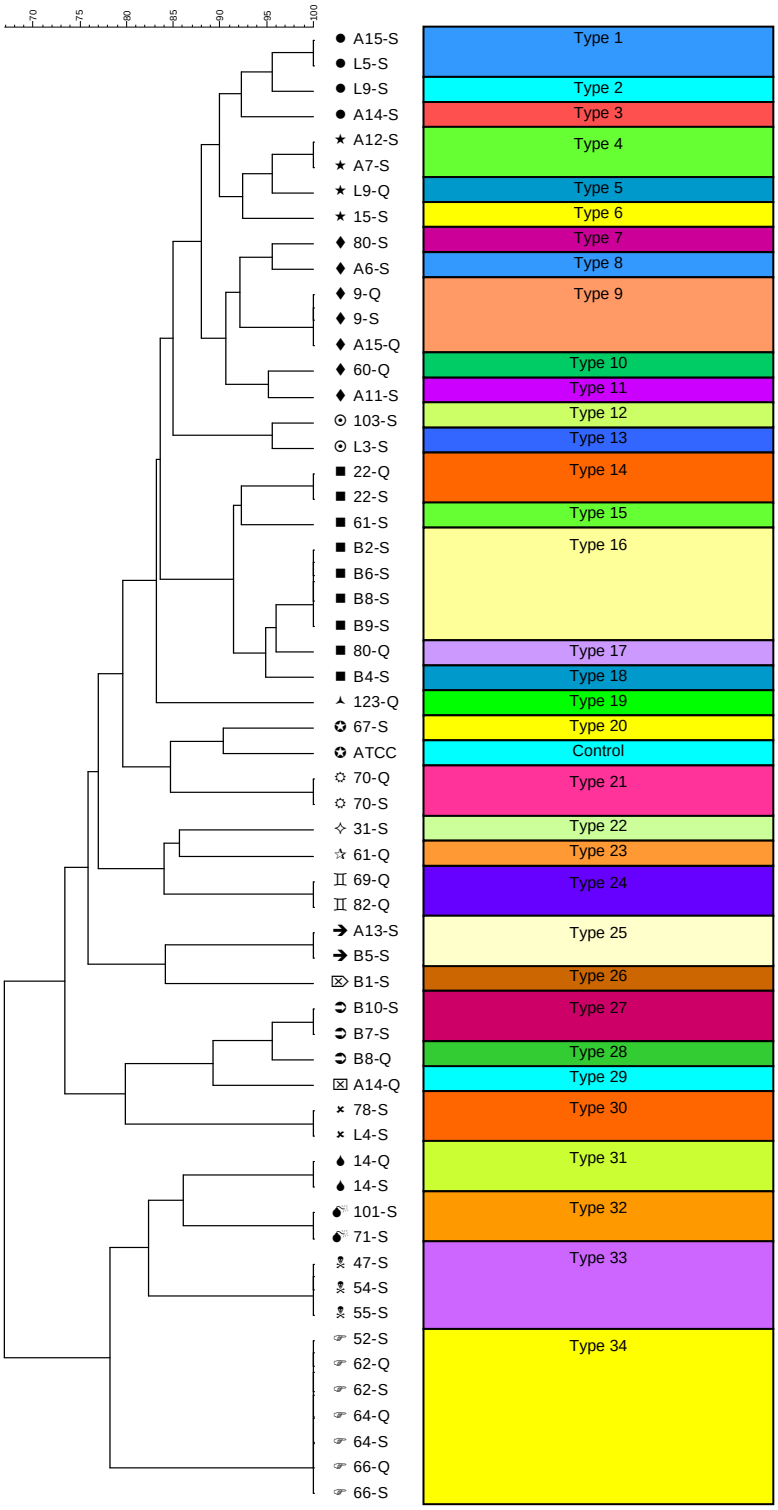

Figure 3.. Phenogram of *E. faecalis* isolates showing *Sma*I-PFGE types at 100% similarity. Symbols to the left of the sample number relate to the similarity of isolates at 90%. Samples with the same symbol show >90% similarity with *Sma*I-PFGE. Isolates are designated 'Q' or 'S' depending on the procedure used to isolate them. 'Q' isolates were refer to those recovered from samples using the quantitation procedure where as isolates designated 'S" refer to those recovered from the enrichment procedure.

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