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Coagulase positive Staphylococci on Australian beef carcases CS.269

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1.0 INTRODUCTION

As a result of the recent survey of the 'Microbiological Quality of Australian Meat' (CS.196), also referred to as the Baseline Survey, two areas of concern were identified, one of which was the high incidence of coagulase positive staphylococci on beef carcasses. Coagulase positive staphylococci were isolated from 28.9% of beef carcasses analysed, 29.3% and 18.8% from export and domestic markets, respectively. This incidence is in marked contrast with the USA, where a detection rate of 4.2% is reported, and also supports reports from Japan of the detection of these bacteria on Australian meat.

Staphylococci are ubiquitous in nature, although they are mainly found on the skin and mucous membranes of mammals and birds. *Staphylococcus aureus* are important to the food industry as some strains are able to cause a variety of infections in food animals, and others are the cause of human foodborne intoxication. The latter results from the ingestion of food in which enterotoxigenic strains have grown to sufficient levels to allow a toxic dose of staphylococcal enterotoxin to be produced before consumption. *S. aureus* was considered for many years to be the only enterotoxigenic species; however, other species such as *S. hyicus* and *S. intermedius* may also form enterotoxins. It is currently recommended that all coagulase positive staphylococci be considered potential food safety hazards.

This present study was undertaken to investigate the reasons for the high incidence of coagulase positive staphylococci reported in the Baseline Study of Australian Meat , CS196, and potential approaches for their control. The specific aims were as follows:

a) To determine the source of contamination of beef carcasses with coagulase positive staphylococci from slaughter to chilling.
b) To specifically identify the *Staphylococcus* spp. present on carcasses and to assess the public health risk.
c) To identify critical limits of control points for coagulase positive staphylococci on animal carcasses.
d) To recommend strategies for the control of coagulase positive staphylococci on carcasses.

2.0 MATERIALS AND METHODS

Determination of the source of contamination of beef carcasses with coagulase positive staphylococci

Selection of abattoirs

Three beef slaughtering establishments were selected according to their performance in the Baseline Survey At each abattoir, the practices along the slaughter line were observed, and in-line sampling points were chosen to provide an indication of the processes which might influence the presence and numbers of coagulase positive staphylococci.

Hide and carcass sampling

Each abattoir was visited on at least three (3) occasions. Hides were sampled immediately following stunning and carcasses were sampled at in-line points determined during the observations at each abattoir (Appendix 1). A 25 cm^2 area of the hide was swabbed with a dry sterile swab, as the animal's hide was wet following washing prior to stunning. A 10 cm^2 sample was excised from dressed carcasses using sterile stainless steel borers from the round, brisket as in the Baseline Survey. Ten hides were swabbed, and 10 round, brisket and flank excision samples were collected during in-line sampling on each visit. At least 30 samples were collected from each of the sites at each of the works with the exception of site 6 (week-end chilling) at plant B where only 20 samples were collected. The round, brisket and flank samples from each carcass were pooled for analysis. It was not feasible to collect samples from the same carcass along the slaughter line; however, samples were collected from animals within the same mob of cattle.

Air and water sampling

Air samples were collected by placing plates of Baird Parker agar (Oxoid CM275) with the lids removed at points in the plant where aerial contamination was likely to occur. Plates were left *in situ* for up to one and processed as for the carcass samples above. Water samples were collected aseptically in sterile bottles and transported to the laboratory chilled. A 200 µl aliquot was spread on Baird Parker agar plates and processed as for the carcass samples.

Swabbing of hands of workers

Each worker was given a moist sterile cellulose sponge, and was asked to thoroughly rub their hands. The worker then placed the sponge in a sterile bag which was transported to the laboratory and processed as for the carcass samples.

Isolation and identification of coagulase positive staphylococci

Samples were analysed for coagulase positive staphylococci using the method of the Australian Standards Association AS766.2.4. Briefly, appropriate dilutions were spread on Baird Parker agar plates and incubated at 37^0C for up to 48h. Typical colonies were selected and tested for coagulase production using the tube

coagulase method (AS766.2.4). It was initially observed that a wide range of colonial types were present on isolation plates from hide samples, many resembling coagulase positive staphylococci. Selected colony types were further identified to ensure accurate counting of presumptive coagulase staphylococci for subsequent samples. As species of *Staphylococcus* other than *S. aureus* can produce coagulase, isolates were tested for additional phenotypic markers to confirm whether the coagulase positive staphylococci were *S. aureus*. These tests included fermentation of mannitol, production of acid aerobically from mannose, maltose and mannitol on MSA (mannitol salt agar), production of acetoin (Vogues-Proskauer test), β-galactosidase activity (ONPG test), and the oxidative and fermentative utilisation of glucose (Hugh and Leifson).

Staphylococcal enterotoxin production

Staphylococcal enterotoxin production was determined using an ELISA (Tecra Diagnostics, Australia). Isolates were grown in brain heart infusion broth at 37^{0}C and the culture supernatants collected and tested using the ELISA according to the manufacturer's instructions, including the appropriate positive and negative controls. Individual enterotoxin types were not determined.

Pulsed-field gel electrophoresis (PFGE) typing

PFGE typing of coagulase positive staphylococcus isolates was performed following a modified method of Smith and Cantor (1987). Briefly, 10 ml of tryptone soy broth (TSB) was inoculated with a single and incubated at 37^{0}C until the OD_{590} reached 0.5. The cells were pelleted, washed twice with PIV buffer (10mM Tris-chloride [pH 7.6], 1M NaCl), and were resuspended in 500 μl of PIV. The cells were warmed to 37^{0}C, mixed with 500 μl of 2.4% PFGE agarose (Bio-Rad), and were dispensed into plug moulds. Chromosomal DNA was prepared by cell lysis in EC lysis buffer (6mM Tris-chloride [pH 7.6], 1M NaCl, 100 mM EDTA, 0.2% Na-desoxycholate, 0.5% sarkosyl), 10 μl of lysozyme (1 mg/ml), and lysostaphin (50 μg/ml), overnight at 37^{0}C, followed by incubation at 50^{0}C overnight in ESP buffer (0.5 M EDTA [pH 9-9.5] containing 1% sarkosyl and 1 mg/ml proteinase K. Plugs were washed four times in TE buffer (10 mM Tris, 1 mM EDTA [pH 8.0]). Approximately 1/3 of a plug was used for DNA digestion. Plugs, pre-incubated in restriction buffer, 1 hr at 25^{0}C, were incubated 20 μl of digestion buffer (38 μl of restriction endonuclease, 260 μl 1 x restriction buffer, 2.6 μl bovine serum albumin) at 25^{0}C overnight. The restriction endonuclease *Sma 1* (Progen Industries, Australia) was used initially to type the selected isolates. Isolates which had indistinguishable PFGE patterns were further tested using *Ksp 1* (Progen Industries, Australia). The DNA restriction fragments were separated in a 1.5% PFGE agarose gel (Bio-Rad) for 2 to 8 s for 12 hr and 10 to 15 s for 12 hr in 0.5 xTBE. The gel was stained with ethidium bromide and visualised using a UV transilluminator.

Growth of coagulase positive staphylococci during chilling

Growth in broth

Preliminary studies were performed to establish growth curves of coagulase positive staphylococcus isolates at 7^{0}C and 10^{0}C in TSB. Coagulase positive staphylococci,

isolated from carcasses both before and after chilling at Plants A, B and C, were inoculated into TSB at a level of approximately 10^6 cfu/ml and incubated with shaking at 9.2^{0}C. The absorbance of each culture was measured at 590 nm at timed intervals during growth. The purity of the inoculum was checked prior to and following the experiment by plating the inoculum onto BHIA, incubated at 25^{0}C. Generation times were estimated from the log of the absorbance values using the model of Baranyi *et al.* (1993).

Growth on beef carcass

Isolates which demonstrated the fastest growth patterns in broth at 10^{0}C were chosen for carcass experiments. A TSB broth culture was diluted to approximately 10^5 cfu/ml and applied to approximately 100cm^2 of the round, brisket and flank areas of a beef carcass to give a final inoculum of approximately 10^3 cfu/cm^2. To approximate the conditions under which staphylococci may be introduced onto the carcass during slaughter, a 'hot' carcass was removed from the slaughter line prior to chilling and inoculated at ambient temperature before being transferred to the chiller. The chiller was set to run a 'normal' week-end chilling cycle. The carcass was held in the chiller for 4 days during which the surface and the air temperature were monitored using thermocouples. A 10 cm^2 sample was excised from the round, flank and brisket, and transferred to the laboratory for immediate enumeration of coagulase positive staphylococci from each sample site, using the methods described previously.

To cover the possibility that coagulase positive staphylococci contaminating carcasses during the slaughter process would be actively growing, the inoculum was also grown to log phase in TSB, in contrast to the stationary phase cultures prepared in the earlier experiment. In addition, the carcass was subjected to "reverse cycle" chilling after holding at 7^{0}C. A 'hot' beef carcass was removed from the slaughter line prior to chilling. The carcass was inoculated at ambient temperature (20-25^{0}C) with coagulase positive staphylococci by painting approximately 100cm^2 of the round and flank areas of the carcass with a suspension of 10^3 cfu/ml. The carcass was transferred to the chiller, adjusted to 7^{0}C, and held for 2 days, after which it was removed from the chiller and held at 25^{0}C for 4 hr to approximate the reverse cycle refrigeration performed in abattoirs prior to boning. Thermocouples were used to monitor air and carcass surface temperature throughout the experiments. A 10 cm^2 area of the round and flank was excised daily, immediately prior to incubation at 25^{0}C, and following 2 and 4 hr at 25^{0}C and coagulase positive staphylococci enumerated using the methods described above.

Statistical analyses

A chi-square test (Microstat, Ecosoft Inc.) was used to compare the incidence of coagulase positive staphylococci obtained at each site and between plants. Significance was defined at the 95% level ($p \leq 0.05$).

Prediction of bacterial growth

The Pathogen Modelling Program (PMP) and the Food Micro Model (FMM) were used to predict generation times of Staphylococci in the above experiments.

3.0 RESULTS

Sampling points within the abattoir

The slaughter practices and in-line sampling points within the three abattoirs were similar (Appendix 1). The major difference was in the method of evisceration. At plants A and B the eviscera was collected into individual trays on a moving bed, while at plant C an individual barrow system was used. All three plants treated the eviscera trays or barrows with an 82^{0}C water wash, followed by cold water rinsing between carcasses. In addition the carcasses were washed with ambient temperature water prior to loading into the chiller at plant A and prior to final trimming at plant C, while at plant B trimming alone was used to remove visible contamination prior to chilling. The hide was sampled following stunning (site 1) while carcasses were sampled prior to head removal (site 2), following evisceration and splitting (site 3), following the final wash at plant A or final trim at plants B and C (site 4), and following overnight (site 5) or weekend chilling (site 6).

Coagulase positive staphylococcal contamination during slaughter and processing

A total of 675 samples were analysed for coagulase positive staphylococci. The incidence and the mean count at each in-line sampling point are shown in Table 1. The overall incidence of coagulase positive staphylococci was 40% for carcasses, and 47% for hides, while the mean count of all samples ranged from 8 to 94 cfu/cm^2.

Identification of coagulase positive staphylococci

A total of 46 isolates from plants A, B and C were further characterised phenotypically. Of these, 37 coagulase positive isolates were identified as *S. aureus* based on their ability to produce acetoin and to utilise mannitol anaerobically. Four isolates were identified as *S. hyicus*, two as *S. intermedius*, and three isolates which were weakly coagulase positive (reaction in 24 hr) closely resembled the coagulase negative species *S. warneri.*

Plant A

Seventy-one percent of hides were contaminated with coagulase positive staphylococci (Table 1). After hide removal 10% of carcasses were contaminated and this increased to 63% immediately following evisceration. The number of contaminated carcasses subsequently decreased with 40% of the carcasses contaminated after the final wash. The number of contaminated carcasses further decreased to 30% after overnight chilling when the average surface temperature of the carcasses, as measured at the shoulder, reached 10^{0}C after 10.5 h chilling (Appendix 2, Fig 2a).

After chilling over a 3 day period, 80% of carcasses were contaminated with coagulase positive staphylococci. Where detected, the mean counts increased from 33 cfu/cm^2 after final wash, to 63 cfu/cm^2 after a 3 day chill (Table 1). During chilling, the surface temperature of the carcasses fell to 10^{0}C after 4.25 h and reached 7^{0}C

after 45 h chilling (Appendix 2, Fig 2b). A summary of the temperature histories for overnight and week-end chilled carcasses is given in Appendix 2.

Plant B

At Plant B, the hides were heavily contaminated and the Baird Parker agar plates were overgrown with other bacteria. After further sub-culture, typical colonies were isolated from 6 samples and only these have been included in the results. After hide removal, 20% of carcasses were contaminated with coagulase positive staphylococci and this increased to 53% after evisceration and to 60% after the final trim (Table 1). The high incidence was maintained after overnight chilling when 60% of carcasses were contaminated and increased during weekend chilling after which 70% of the carcasses were positive. The average number of coagulase positive staphylococci on positive carcasses increased from 27 cfu/cm^2 before chilling to 112 cfu/cm^2 after week-end chilling. After entering the chiller, the time taken for the surface temperature of carcasses to reach 10^{0}C during an overnight and week-end chill, was 17.5 and 22.5 h respectively (Appendix 2, Figs. 2c and 2d).

Plant C

The incidence of coagulase positive staphylococci on the hides of animals slaughtered at plant C was 50% (Table 1). The incidence on carcasses after hide removal was 27%, and this fell to 17% following evisceration and was further reduced to 13% after final trimming. After overnight chilling when the surface temperature of the carcasses fell to 10^{0}C after 13.5 h (Appendix 2, Fig. 2e), the incidence of coagulase positive staphylococci on the carcasses was 13%. During weekend chilling the surface temperature reached 10^{0}C after 39.5 h (Appendix 2, Fig. 2f). The incidence of coagulase positive staphylococci on carcasses chilled over a weekend was 47%. The mean count on positive carcasses was 19 cfu/cm^2 after the final trim, and 15 and 16 cfu/cm^2 after overnight and weekend chilling, respectively.

Table 1. The incidence and mean count of coagulase positive staphylococci of in-line samples of hides and beef carcasses at Plants A, B and C.

Site	Plant A		Plant B		Plant C	
	% +[a]	Count [b]	% +	Count	% +	Count
1	71	29	20	8	50	94
2	10	13	20	16	27	19
3	63	45	53	36	17	11
4	40	33	60	27	13	19
5	30	21	60	25	13	15[c]
6	80	63	70	112	47	16

[a] incidence of isolates (%)
[b] cfu/cm^2. Limit of detection is one (1) colony per plate = 10 cfu/cm^2 for carcasses, and 2 cfu/cm^2 for hides. Counts are the average of positive samples only.
[c] 2/40 samples with high counts of coagulase positive staphylococci were not included.

Identification of critical control points

Hide removal

Coagulase positive staphylococci were frequently detected on the hides of animals prior to slaughter. It is recognised that the process of hide removal will result in some microbiological contamination of the underlying tissue and that the extent of contamination will depend on the technique used and the level of contamination on the hide. Therefore the hide was likely to be the major source of contamination of carcasses sampled immediately after hide removal.

Evisceration

The number of contaminated carcasses and the extent of the contamination increased at plants A and B during the evisceration process, in contrast to plant C where the incidence decreased ($P=<0.02$). The only difference observed between these plants at this sample point was the method of collection of the eviscera eg. a moving bed evisceration table (plants A and B) and a barrow system (plant C).

Final wash and trim

At plant A, after the final carcass wash, the incidence of coagulase positive staphylococci on carcasses decreased from 63% to 40%. At plants B and C, trimming of fat and visible contamination was the final step prior to chilling. There was an increase of 7% in the incidence of coagulase positive staphylococci on carcasses after the final trim at plant B (53% to 60%), and a 4% reduction at plant C (17% to 13%). At plant C, heavy trimming of fat on the slaughter floor was used to help overcome some of the problems of hard fat after chilling.

Chilling

At plants A, B and C, an increase in the number of coagulase positive staphylococci was observed between the final sampling point on the line and the end of weekend chilling (Table 1). The implications of this will be discussed in a subsequent section. In contrast, after overnight chilling at all plants the incidence of coagulase positive staphylococci decreased or remained the same.

To better define the critical control points and control measures, further investigations were carried out at plant A, where the largest increases in contamination occurred. The isolates of coagulase positive staphylococci were typed using pulsed field gel electrophoresis (PFGE) which together with enterotoxin production, was used to determine strain relationships and potential sources of contamination. Experiments were performed to determine the behaviour of coagulase positive staphylococci isolated from the plant during chilling.

Evisceration at Plant A

A high incidence of contamination was demonstrated at three points at Plant A, on the hide, on carcasses after evisceration, and on carcasses following a weekend chill.

Round, brisket, and flank samples were taken from 15 carcasses prior to and immediately after evisceration, and were analysed separately for staphylococci. Before evisceration, coagulase positive staphylococci were not detected on any of the carcasses; however, after evisceration, 63% of the carcasses were found to be positive. Differences in the distribution of contamination at the three sites were detected (Table 2). The incidence of contamination increased on the brisket and the flank, while remaining low on the round. Both of the former sites were observed to be handled by the evisceration workers during this operation.

Table 2. Incidence of coagulase positive staphylococci at three sampling sites on carcasses at plant A.

Dressing operation	Incidence at carcass sites (%)		
	brisket	round	flank
before evisceration	0	0	0
after evisceration	30	5	40

The hands of three workers (designated A, B and C) eviscerating carcasses were swabbed before and during work, at the same time the carcasses were sampled before and after evisceration (Table 3). The hands of the three workers were heavily contaminated with coagulase positive staphylococci. Workers A and C had high numbers of these bacteria prior to commencing work although the numbers decreased during work. The counts on the hands of these workers increased slightly after washing. On the hands of worker B, the number of staphylococci increased after working for an hour and then decreased 10 fold after hand washing. Carcass isolates collected after evisceration, during the same period that the workers hands were sampled, all produced staphylococcal enterotoxin.

Isolates were obtained from other abattoir workers operating at various points downstream of evisceration, as well as from staff not associated with the handling of carcasses eg clerical staff. All trimming staff tested carried coagulase positive staphylococci on their hands. With the exception of one isolate (ground staff), all non-meat worker isolates were coagulase negative.

Table 3. Counts of coagulase positive staphylococci present on the hands of the evisceration workers at Plant A.

Worker	Sample	cfu/hand
A	Before work	2.0×10^4
	After one hour	1.2×10^3
	After washing hands	1.3×10^3
B	Before work	3.5×10^2
	After one hour	1.4×10^4
	After washing hands	1.7×10^3
C	Before work	5.8×10^3
	After one hour	2.0×10^1
	After washing hands	2.3×10^2

Staphylococcal enterotoxin production

One hundred and fifty-five carcass isolates from plants A, B and C and nine environmental isolates from Plant A, which exhibited typical *S. aureus* colony morphology on Baird Parker medium, were tested for enterotoxin production. Enterotoxin was detected from 61% of the total isolates (Table 4). The greatest percentage of isolates producing enterotoxin were detected following evisceration and after final trimming or washing (sites 3 and 4), while only a small number of water and air isolates were tested, all were found to produce toxin.

Table 4. Enterotoxin production of coagulase positive staphylococci isolated from beef carcasses, water and air at Plants A, B and C.

Site of isolation	No. of isolates	No. enterotoxin positive	No. enterotoxin positive (%)
1	26	9	35
2	8	4	50
3	36	29	81
4	6	5	83
5/6	79	47	59
Water	7	7	100
Air	2	2	100

Pulsed field gel electrophoresis (PFGE) typing of coagulase positive staphylococci

To further define the source of contamination at plant A, coagulase positive staphylococci isolated from sites 1, 2 and 3, were typed using PFGE. A total of 31 isolates collected prior to evisceration and 25 isolates collected after evisceration were typed (Table 5). Thirteen PFGE patterns were detected and have been arbitrarily given alphabetical designations.

Table 5. Distribution of PFGE types and enterotoxin production of coagulase positive staphylococci isolated before and after evisceration at plant A.

Before evisceration			After evisceration		
PFGE type	Toxin[b]	% Isolates	PFGE type	Toxin	% Isolates
C	-	40	I	+	77
B	-	24	K	+	7
K2[a]	-	12	L	-	7
G	-	8	I(+)	+	3
E	-	4	J	+w	3
A	-	4	M	+w	3
E1	-	4			
H	-	4			

[a] Pattern not shown in Appendix 3. [b] Toxin production

The predominant PFGE pattern of isolates collected before evisceration (type C) was different from the predominant pattern of isolates collected after evisceration and

carcass splitting (type I). The main PFGE pattern of isolates from evisceration workers hands and from carcasses slaughtered on the same day was type I (Table 5 and Appendix 3). All isolates collected from non-evisceration abattoir personnel and clerical staff, demonstrated PFGE types distinguishable from those of isolates collected from carcasses after evisceration and from the hands of evisceration workers.

PFGE was used to type isolates obtained both before and after evisceration at plant B (Table 6). There was no predominance of any single PFGE type among isolates obtained before evisceration. Type J was the predominant PFGE type among isolates from carcasses after evisceration..

Table 6. Distribution of PFGE types of coagulase positive staphylococci isolates collected before and after evisceration at plant B.

PFGE type	No. isolates (%)	
	Before evisceration	After evisceration
A		-[a]
A1	8	-
C1	-	7
F1	8	-
I	16	7
J	8	43
J1	-	7
K	16	14
K1	8	-
M	8	14
Z	-	7

[a]- not detected

At plant C, PFGE type A predominated among isolates from carcasses before evisceration (Table 7) while type K was the predominant type after evisceration.

Table 7. Distribution of PFGE types of coagulase positive staphylococci isolates collected before and after evisceration at plant C.

PFGE type	No. isolates (%)	
	Before evisceration	After evisceration
A	25	8
F1	8	-[a]
I	8	8
K	17	33
M	-	8
N	8	-
O	8	-
P	8	17
R	17	-
T	-	8
U	-	8

[a] - not detected

The PFGE types were not restricted to isolates collected either before or after evisceration.

Chilling of carcasses

In the three plants, the incidence of contaminated carcasses increased following weekend chilling (Table 8). A concomitant increase in the number of coagulase positive staphylococci was observed for Plants A and B. The count did not increase significantly at Plant C.

Table 8. Incidence and count of coagulase positive staphylococci at Plants A, B and C before and after chilling.

Plant	Site	Incidence (%)	Mean count (cfu/cm^2)
A	4	40	33
	5	30	20.8
	6	80	63.4
B	4	60	26.3
	5	55	24.6
	6	70	112.00
C	4	13	18.6
	5	13	15[a]
	6	40	16.1

[a] Two out of forty samples with high counts of coagulase positive staphylococci were not included.

Growth of coagulase positive staphylococci at refrigeration temperatures

Growth of coagulase positive staphylococci - use of predictive models

The Food Micro Model was used to predict growth rates of staphylococci at various temperatures. This data, with the incidence and numbers of coagulase positive staphylococci, and surface temperatures during chilling, were used to approximate the expected increase in the numbers of these bacteria during week-end chilling (Table 9). The mean count reported in Table 9 was obtained by multiplying the mean count for positive carcasses by the incidence of positive carcasses to give an adjusted mean which more closely represents the true distribution of staphylococci on the carcasses.

The predicted increases in coagulase positive staphylococci at plants A and B were similar to those observed during in-line sampling. However for plant C the prediction model used did not agree with the observed increase.

Table 9. Counts of coagulase positive staphylococci on carcasses sampled before and after chilling at plants A, B, and C, with predicted increases.

Site	Mean Count log cfu/cm^2	Increase	
		Mean log cfu/cm^2	Predicted log cfu/cm^2
Plant A			
Before Chilling			
After Overnight Chill	0.61	.[a]	.
After week-end Chill	0.39	0.22	.
	1.44	0.83	0.74
Plant B			
Before Chilling			
After Overnight Chill	0.85	.	.
After week-end Chill	0.83	(-0.02)	.
	1.43	0.58	1.14
Plant C			
Before Chilling			
After Overnight Chill	0.17	.	.
After week-end Chill	0.30	0.13	.
	0.56	0.39	2.95

[a] not determined

Growth of coagulase positive staphylococci in laboratory media

(1) Growth at 10^{0}C

The ability of coagulase positive staphylococci to grow at 10^{0}C (average temperature 9.2^{0}C) in TSB was investigated. Generation times were calculated for 10 isolates and these values are shown in Table 10. Fourteen coagulase positive staphylococci isolated from carcasses both before and after chilling were able to grow and three did not.

grow at 9.2^{0}C in TSB.

(2) Growth at 7^{0}C

Isolates actively growing at 10^{0}C did not grow over a four week period when transferred to TSB and incubated at 7^{0}C.

Growth of staphylococci isolates on a beef carcass under simulated chilling conditions

(1) Growth at 10^{0}C

Four isolates of coagulase positive staphylococci, previously shown to exhibit the fastest generation times in TSB at 10^0C, were grown to stationary phase, inoculated onto the surface of a beef carcass and held at 10^0C for 3 days.

Isolate 6a (Table 9) was the only isolate which grew on the surface tissue during the course of the experiment, with growth only observed on the brisket. The generation time for staphylococci, based on the actual count obtained during chilling, was estimated to be between 4 and 9 h. The PMP and FMM estimate a generation time of 7 h and 10 h, respectively, for staphylococci growing at 10^0C, therefore the observed generation time is close to that estimated by the two models. During the four day chilling cycle, the counts of this strain increased 2 log units (100 fold) from the time of inoculation, with an observed lag of two days. During the three days of a normal week-end chilling cycle the numbers of staphylococci increased 0.7 log units.

(2) Growth at 7^0C

Two coagulase positive staphylococci, previously shown to grow in TSB at 10^0C, were inoculated when in stationary phase onto the round, brisket and flank surfaces of a beef carcass. The temperature recording showed that the holding temperature, after the initial chilling phase, averaged 7^0C; however, neither of the strains grew during the course of the experiment (72 h).

Table 10. Generation times of coagulase positive staphylococci at 9.2^0C on a beef carcass.

Plant	Isolate	Site of isolation	Toxin[a]	Growth	Generation time (hr)
A	1a	Before chilling	-[b]	-	-
	2a		-	-	-
	3a		+[c]	+	59
	4a	After chilling	+	-	-
	5a		+	+	ND[d]
	6a		+	+	56
B	1b	Before chilling	+	+	49
	2b		+	+	47
	3b		+	+	ND
	4b	After chilling	+	+	ND
	5b		+	+	73
	6b		+	+	73
C	1c	Before chilling	+	+	55
	2c		+	+	52
	3c	After chilling	+	+	ND
	4c		+	+	60
	5c		-	+	37

[a] staphylococcal enterotoxin production [b] positive [c] negative
[d] growth rate not determined

Growth of staphylococci on a beef carcass under chilling conditions simulating an abattoir weekend chill and reverse cycle chilling

The isolates used in this experiment had been used in previous trials; however, in this study cultures were subsequently grown to log phase (to reduce any lag) and inoculated onto the round and flank surface of a beef carcass. No appreciable difference in growth patterns was observed between the three isolates; therefore, the data for each strain were pooled for each inoculation site.

No growth was observed on the round or flank during the initial 24 hr chill, during which time the carcass surface temperature fell from an initial temperature, at the time of inoculation, of 20^{0}C to 5^{0}C. After 24 h, the chiller temperature was adjusted to approximately 7^{0}C and the carcass was held at this temperature for a further 2 days. During this time, little if any increase in the numbers of staphylococci was observed on the round; however, a 0.2 log (1.6 fold) increase was observed on the flank.

Following the chilling cycle, the air temperature was increased to 25^{0}C for approximately 4 hr, to represent reverse cycle refrigeration prior to boning. A small increase in the numbers of staphylococci was noted during this time (0.1-0.2 log), however the increase is unlikely to be significant.

4.0 DISCUSSION

The major reservoirs of coagulase positive staphylococci are humans and animals. The contamination of beef carcasses may originate from the animals, the handlers during slaughter and subsequent handling and contact surfaces. The carriage rate of coagulase positive staphylococci among healthy humans in the general community is estimated to be between 30 and 50% at any one time. Some reports of the carriage of staphylococci among food handlers are higher. Bergdoll (1989) reported staphylococcal isolation rates from food handlers in Germany and Japan of 36.9 and 99%, respectively. De Witt and Kampelmacher (1981) reported 65 to 100% of abattoir workers hands were contaminated with *S. aureus*.

Bovines are considered an important animal food source of coagulase positive staphylococci; however, this is mainly because staphylococci are a cause of mastitis as the bacterium or toxins may be transmitted in milk (Bergdoll, 1979). In 1972, Fomin and Simmons surveyed boneless beef in North Queensland and detected coagulase positive staphylococci in 88% of 92 samples. Subsequently, this group determined the presence of coagulase positive staphylococci at various sites in an abattoir slaughtering cattle for boneless beef (Peel, *et al.*, 1975). The number of samples tested was small, although a trend was observed where the incidence of coagulase positive staphylococci increased as processing progressed.

In this study, the overall incidence of coagulase positive staphylococci detected on beef carcasses from three abattoirs was 34 and 65%, after an overnight and three day chill, respectively. This confirmed the high counts previously reported in the larger Survey of the Microbiological Quality of Australian of Meat, CS196. Following observation of the flow diagrams of the slaughter and chilling processes in three abattoirs, six sites were chosen which would allow the identification of any processes where the contamination of carcasses with coagulase positive staphylococci was occurring and where the numbers of coagulase positive staphylococci were increasing or decreasing.

The hides of animals at slaughter were heavily contaminated, 71% and 50% at Plants A and C, respectively, and the process of hide removal was the initial site of carcass contamination. Compared with other sites on the line, the incidence of positive carcasses (10 to 27%) and the counts of coagulase positive staphylococci (13-27 cfu/cm^2) , at this point were comparatively low. The hide contamination is similar to the observation of Peel *et al.* (1975) who reported 77.5% of 40 hides positive.

The carcass contamination rate and contamination levels significantly increased at Plants, A and B, and decreased at Plant C during evisceration. The only observed difference between the Plants was the moving bed system for the removal of the eviscera at Plants A and B and a barrow system used at Plant C. A detailed study was not undertaken to determine whether this difference had some relevance to the contamination. In addition, the spatial design of the plant and aerial contamination were not examined. Contact with the workers hands was common at all plants at this point. At plant A, contamination of the carcasses during evisceration was occurring at the flank and brisket where the greatest contact was observed with worker's hands and not at the round which was less accessible.

After evisceration, carcasses were either washed or trimmed before transfer to the chillers. At plant C carcasses were heavily trimmed of fat to aid in chilling which resulted in a small decrease in the incidence of coagulase positive staphylococci. This is in contrast to plant B, where only visible contamination was removed during trimming and a small increase in incidence was noted. Plant A used trimming followed by washing to remove visible contamination and achieved a 20% decrease in the incidence of staphylococci. During trimming, variable amounts of contact of the worker's hands over a carcass and between carcasses was observed and this could result in contamination with the indigenous flora from the operator's hands or carcass cross-contamination via knives and hands. These sources were not specifically investigated here, although Peel *et al.* (1975) found high counts and widespread distribution of coagulase positive staphylococci on workers hands, mesh gloves and aprons, knives and saws in the slaughter area.

In these abattoirs, it was concluded trimming of visible contamination did not reduce the presence of staphylococci and may increase contamination slightly. Hot fat trimming similar to that practiced at plant C has been shown, in other studies, to be ineffective in reducing the total bacteria present on carcasses surfaces (Prasai *et al.*, 1995). Trimming has been shown to be effective in removing faecal material when performed correctly (Regan *et al.*, 1996), resulting in lower total and *Escherichia coli* counts at artificially contaminated sites. No reports on the effect of trimming on the numbers of staphylococci were found.

To determine the source of contamination and transmission of coagulase positive staphylococci during slaughter, the relationships between the isolates from the various sites and workers were determined based on their ability to produce staphylococcal enterotoxin and by comparison of the patterns obtained from pulsed field gel electrophoresis of *Sma*I restriction digest fragments of their chromosomal DNA. Phage typing has been extensively used in the past ; however, it has limited discriminatory power. Fomin and Simmons (1972) phage typed the isolates of coagulase positive staphylococci isolated from boned meat samples collected from an Australian abattoir and found 71.8% were not typable and 23% contained phage types associated with human sources of *S. aureus*.

Typing of the isolates indicated that contamination was occurring during hide removal while further types were being introduced at this point, possibly via the workers. At Plants A and B, the different PFGE types and enterotoxin production of isolates indicated significant contamination was being introduced during the evisceration process. At Plant A, a major source was the worker's hands and water and air in this area were also contaminated. Further contamination was possible from operators downstream from the evisceration station, as they were also observed handling the carcasses, although the same types were not detected on carcasses and their hands. The sites on carcasses in contact with the worker's hands at this station were different from the original carcass sampling sites and this may have influenced this result.

It is proposed that meat workers, at least at plant A, are predisposed to colonisation by staphylococci. The skin of the hands of the evisceration staff at plant A were in poor condition and counts of 10 to 10^4 cfu coagulase positive staphylococci were

isolated per hand, prior to commencing work and after an hour of work. The nature of the work performed by these workers is likely to contribute to this condition, with the evisceration worker's hands carrying higher numbers of the bacterium than those workers downstream. In contrast, site staff not involved in the slaughter operations had a low incidence of staphylococcal colonisation. When the workers washed their hands the counts did not change significantly. Washing hands with soap alone does not eliminate these organisms and the use of disinfectants during washing or applied to clean dry hands has been recommended (Henzel, 1984); however, further studies in this area are required. These findings are similar to those reported by Isigidi *et al.* (1990) who isolated biotypes of *S. aureus* from meat products which were similar to those isolated from the anterior nares of abattoir workers, although rarely found in persons not in frequent contact with meats.

Overnight chilling of carcasses was effective in controlling the growth of coagulase positive staphylococci. In contrast, weekend chilling resulted in increased carcass contamination rates in each of the Plants in the study. This contrasts with the report of Saide-Albornoz *et al.* (1995) who observed a linear increase in the numbers of *S. aureus* on pork from slaughter to fabrication, with the highest numbers being detected after 24 h chilling below 10^0C. Peel *et al.*, 1975, detected coagulase positive staphylococci on 50% carcasses sampled in the chillers in an Australian abattoir; however, the duration of chilling was not stated. In this study ,the increase may have been due to resuscitation and/or growth of contamination introduced prior to chilling or that resulting from cross-contamination between carcasses and from workers in the chillers.

The optimal growth temperature of staphylococci is 35-37^{0}C, with growth reported in some foods at temperatures as low as 6.7^{0}C (Bergdoll, 1989). In this study, staphylococci isolated before and after chilling at the three plants, grew in laboratory media at 10^{0}C and failed to grow at 7^{0}C. Carcass cooling relies on both temperature reduction and surface drying to inhibit microbial growth. The ability of the staphylococci to grow at refrigeration temperatures is of concern as they are also capable of growing over a wider range of water activity (a_w) than common aerobic spoilage bacteria found on carcasses. The reduced a_W of the surface tissue during chilling may select for the growth of staphylococci, which are otherwise poor microbial competitors.

Experiments where beef carcasses were artificially inoculated and chilled under controlled temperature conditions, confirmed the observations in the abattoirs. Little if any growth in staphylococci occurred on the carcass during the first 24 h of chilling when the bacteria are likely adapting to the conditions on the meat surface. Subsequently, holding carcasses at or above 10^{0}C could lead to growth of staphylococci at some sites on the carcass. The amount of growth observed here, after a two day lag, was small (0.7 log increase) but similar to the increase observed over a similar time during the in-line study. The total increase in the numbers of staphylococci observed after four days was approximately 2 log (100 fold).

Staphylococcal foodborne disease is an intoxication resulting from the ingestion of food containing staphylococcal enterotoxin. *S. aureus* is the most common enterotoxigenic staphylococcal species and in this study, 61% of the total isolates produced staphylococcal enterotoxin. Isolates present on the carcasses after

evisceration, in the chillers and on the evisceration workers hands were predominantly enterotoxigenic, in contrast to those on animal hides and carcasses before evisceration. Enterotoxigenic strains are less commonly found in cattle than humans (Bergdoll, 1989) and this finding further supports the proposal that the workers were a major source of contamination in Plant A. Fifty-nine percent of isolates on chilled carcasses were enterotoxigenic which is in close agreement with the proportion of toxigenic isolates (~50%) reported in the Australian Baseline Survey (CS196). Although the isolates were enterotoxigenic, the risk of toxin production is low as staphylococcal enterotoxin is not usually produced at temperatures below 15^{0}C. As growth is much slower at temperatures approaching 10^{0}C, the proportionate amount of enterotoxin produced is low unless the food is held at these temperatures for extended periods.

Raw meats are generally not considered a direct hazard for foodborne disease because staphylococci are considered poor competitors and other bacterial contaminants inhibit their growth in raw products under most conditions. In addition, staphylococci are controlled by common processes used in the preparation of meat products eg. cooking and fermentation. However, raw meat is a potential source of staphylococci for other foods and pre-cooked or preserved meat products via cross-contamination of surfaces, utensils and hands in food preparation facilities. Epidemiological studies indicate the majority of food poisoning outbreaks result from contamination of food, after cooking or preserving by food handlers with some type of staphylococcal infection and subsequent poor control of the time and temperature during storage of the contaminated food (Bergdoll, 1989)

From this study, two points were identified where control could be implemented, preventing contamination of enterotoxigenic staphylococci during evisceration and subsequent handling, and control of the growth of the bacterium during chilling. The former is applicable at plant A and it should be noted that as only three abattoirs were studied, it can not be suggested that this is generic to all abattoirs. A study was planned at plant A to evaluate an intervention to prevent the contamination of carcasses by workers; however, perceived industrial problems led to cancellation of the study. It has been proposed that strict hand washing practices, enforced by management, can be effective in preventing contamination of carcasses by workers (Heinzel, 1984). However the degree of washing necessary to ensure adequate removal of bacteria from hands was excessive and would not be practical for use between carcasses on a beef processing line operating at chain speeds observed. The use of gloves was considered in the proposed study at Plant A, although it was recognised that the occupational safety issues of this approach would have to be addressed. The use of sanitisers may provide a means to reduce the numbers of staphylococci contaminating workers hands although these have to be of an appropriate formula for use in a food for human consumption. Treatments which will also improve the condition of the workers hands are desirable and should be considered. Further research in this area is needed to determine practical, feasible and sustainable intervention strategies for the meat industry.

The need for the control of the temperature of carcasses during chilling was common to each Plant studied. When holding temperatures were reduced to 7^{0}C little, if any, growth of coagualse positive staphylococci occurred during chilling in agreement with published data on the minimum growth rate for staphylococci. A trial was

performed at Plant C, where carcasses were held at 7^0C for 48 h. Problems were encountered as carcasses were unable to be boned due to 'hard fat'. Current AQIS regulations allow the surface of beef carcasses, once cooled, to rise above 10^0C for a period not exceeding 3 h. In the present study, surface rewarming, as used in 'reverse cycle refrigeration', was evaluated before boning and little increase in the numbers of staphylococci occurred during this chilling regime. Previous work (unpublished) has shown that similar treatment of beef carcasses inoculated with *E. coli* resulted in no increase in the numbers of these bacteria. However, it is essential for the control of bacterial growth that the surface of the carcass is cooled rapidly to $<7^0$C and held at this temperature until reverse cycle refrigeration is applied.

In conclusion, the contamination of carcasses with coagulase positive staphylococci, particularly by workers during slaughter operations eg evisceration and trimming, should be prevented and warrants further attention. The control of carcass chilling is essential for the control of the growth of coagulase positive staphylococci together with other bacterial pathogens on carcasses.

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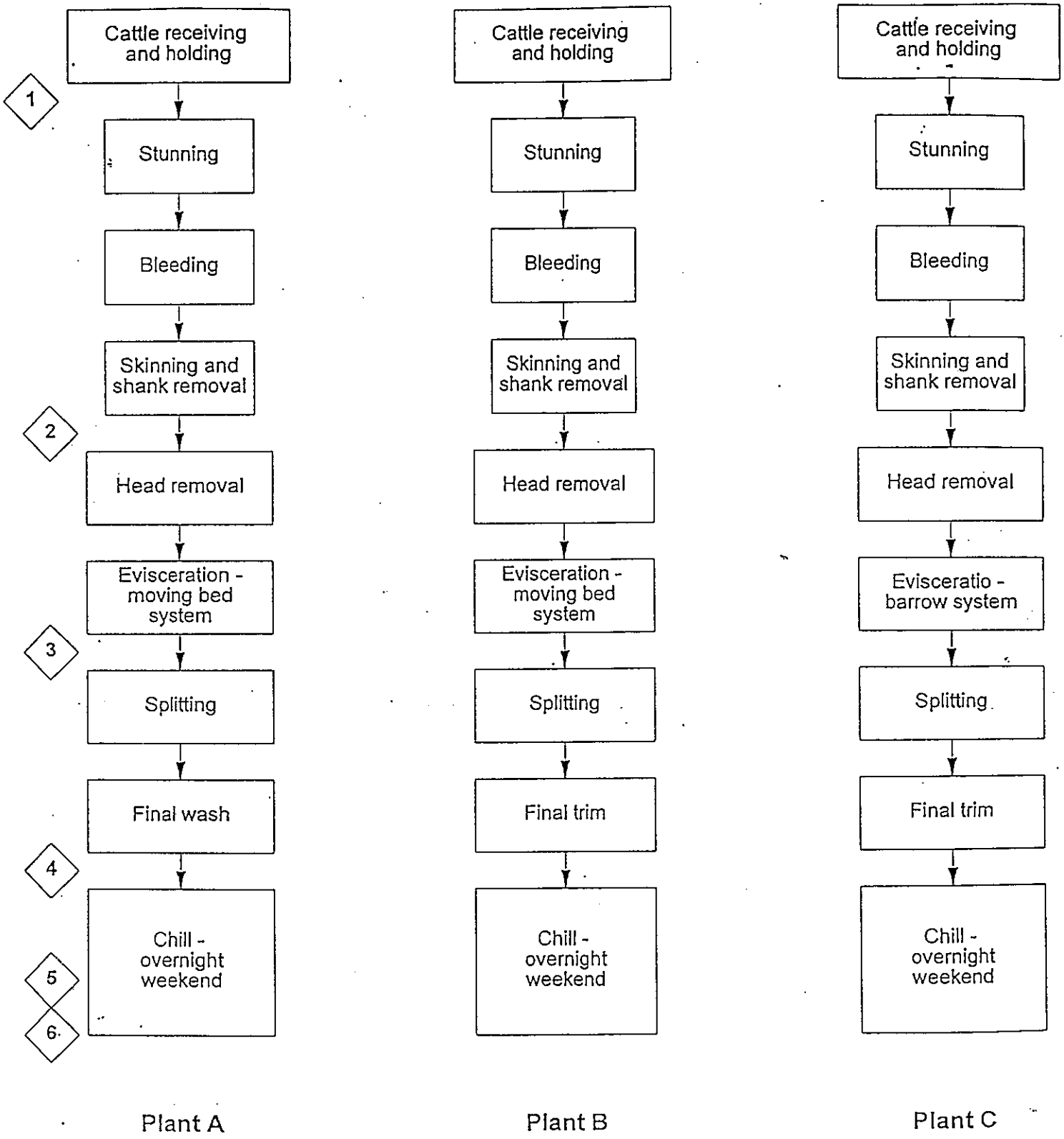
APPENDIX 1 Flow diagram of slaughter of beef carcasses at Plants A, B and C and sampling sites for coagulase positive staphylococci.



◇ Sampling points

APPENDIX 2 Temperature recordings during chilling experiments

Summary of temperature histories for carcasses chilled at plants A, B, and C

			Time to reach temperature at specific carcass sites (hr)		
	Av. weight (kg)	Set air Temp.[a] (^{0}C)	Deep muscle to 20^{0}C	Surface to 10^{0}C	Surface to 7^{0}C
O/night					
A	192	6-7	14.5	4.25	NR [b]
B	166	6-8	17.5	18.5	NR
C	154	2-3	13.5	8.5	NR
Week-end					
A	181	6-7	13	10.5	45
B	151	8-9	22.5	16.5	NR
C	208	9-9.5	18.5	39.5	NR

[a] Holding temperature

[b] NR - Temperature not reached

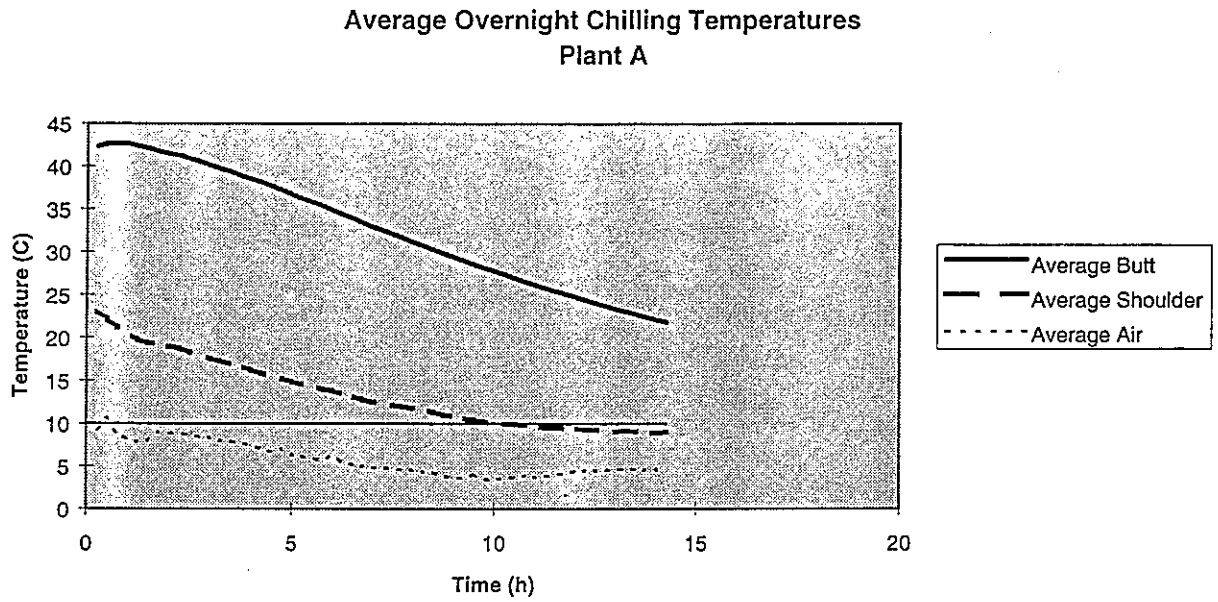


Figure 2a: Average overnight chilling temperatures recorded at Plant A

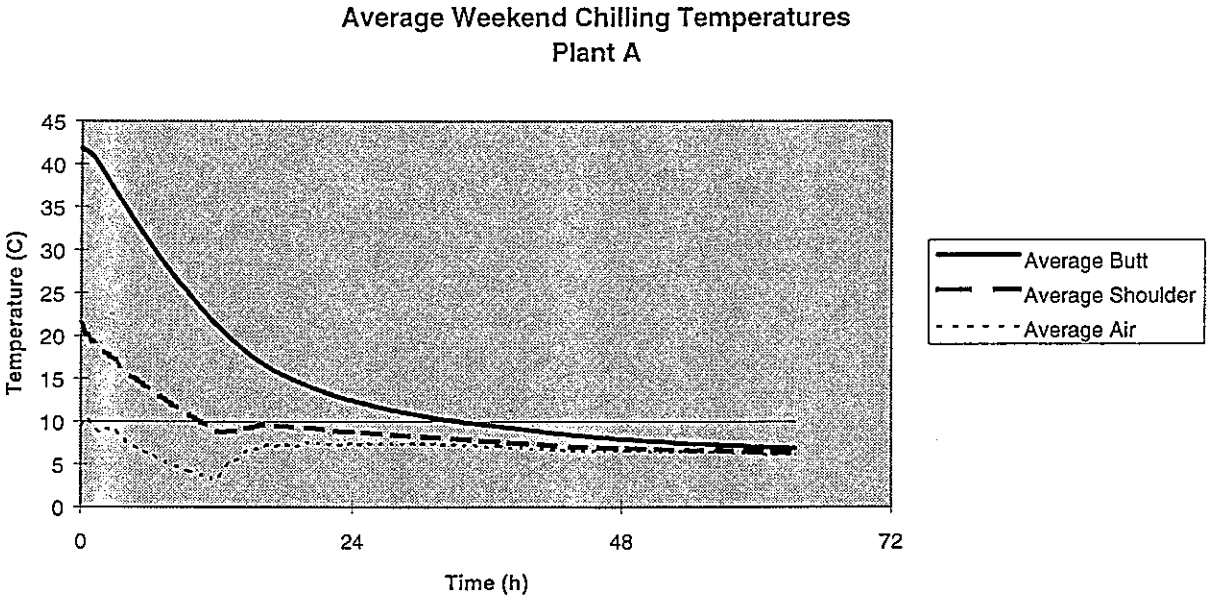


Figure 2b: Average weekend chilling temperatures recorded at Plant A

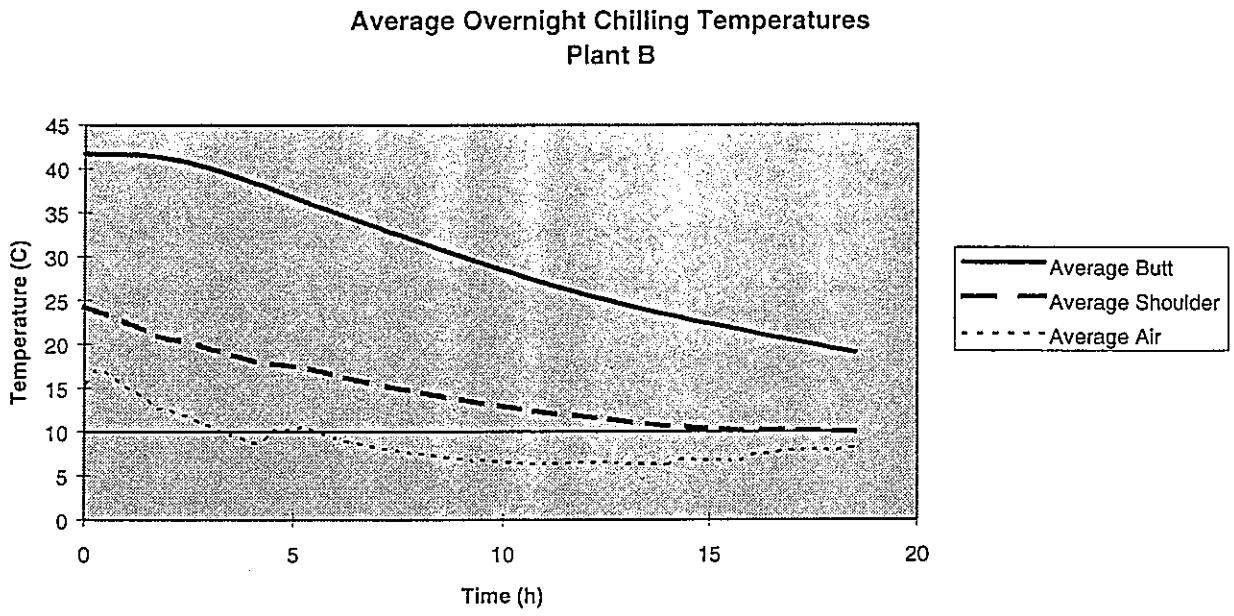


Figure 2c: Average overnight chilling temperatures recorded at Plant B

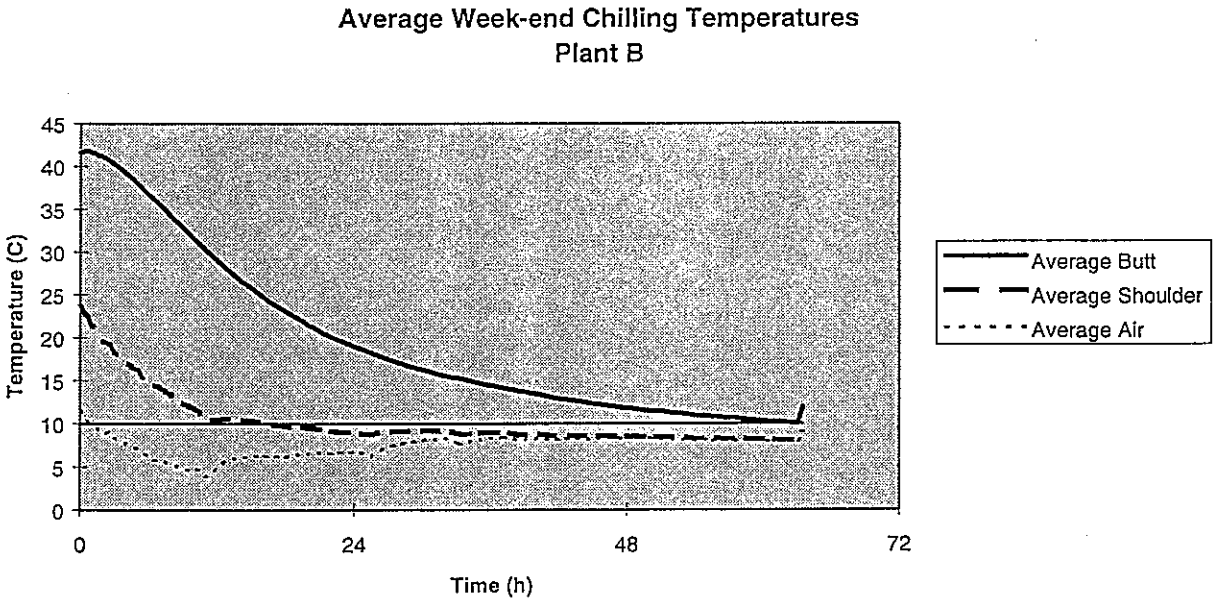


Figure 2d: Average weekend chilling temperatures recorded at Plant B

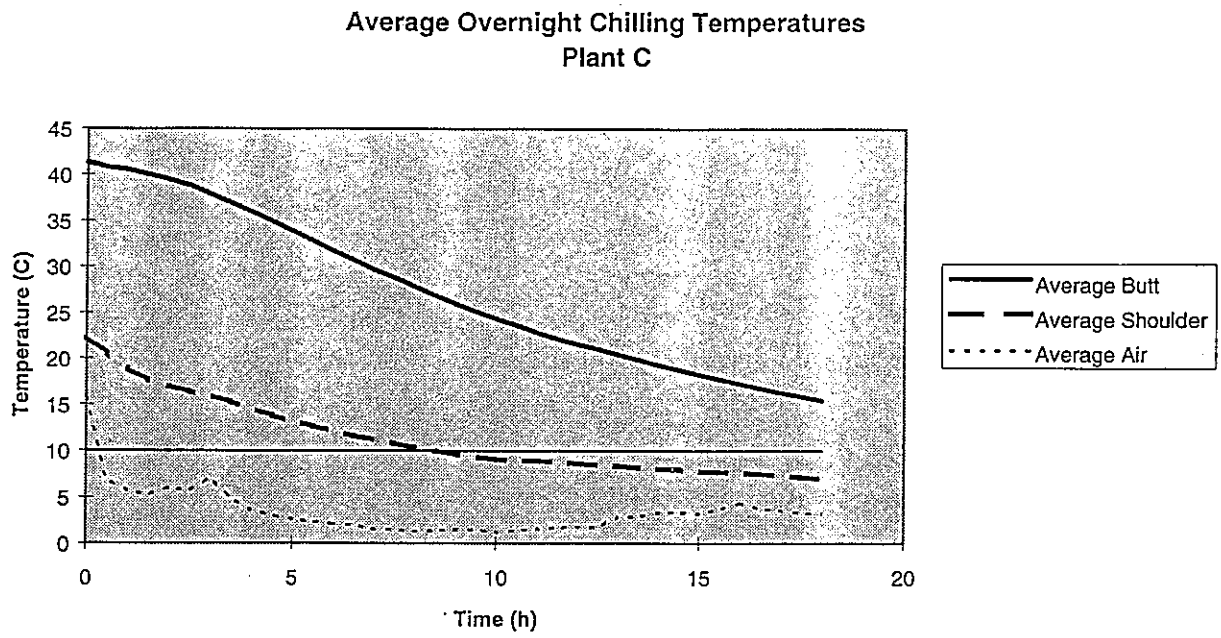


Figure 2e: Average overnight chilling temperatures recorded at Plant C

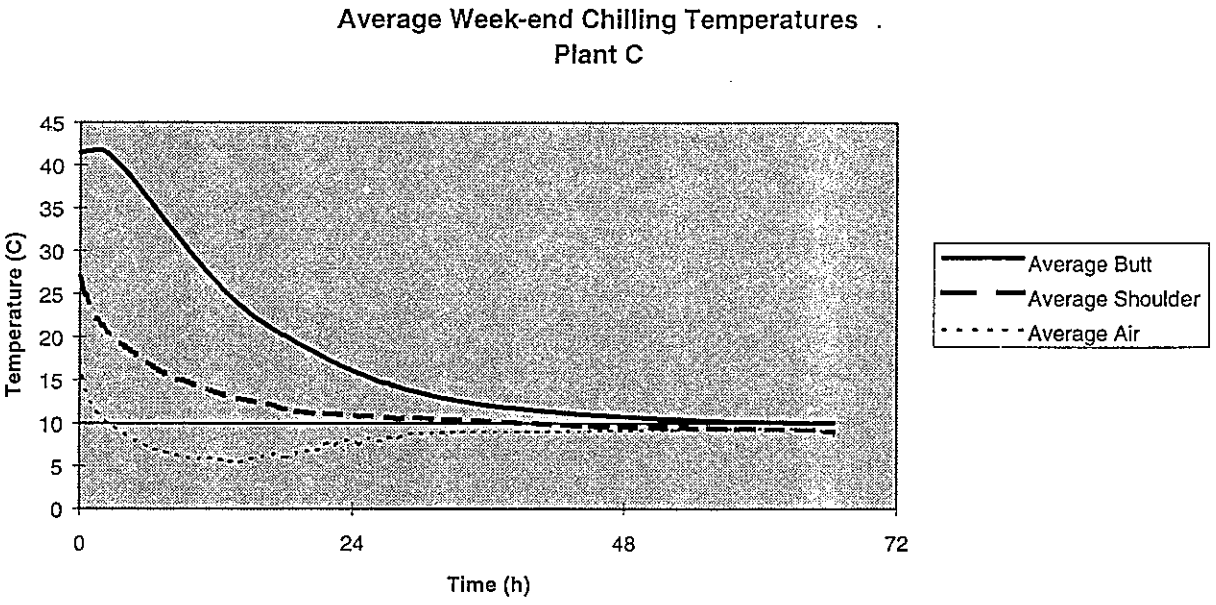


Figure 2f: Average weekend chilling temperatures recorded at Plant C

APPENDIX 3

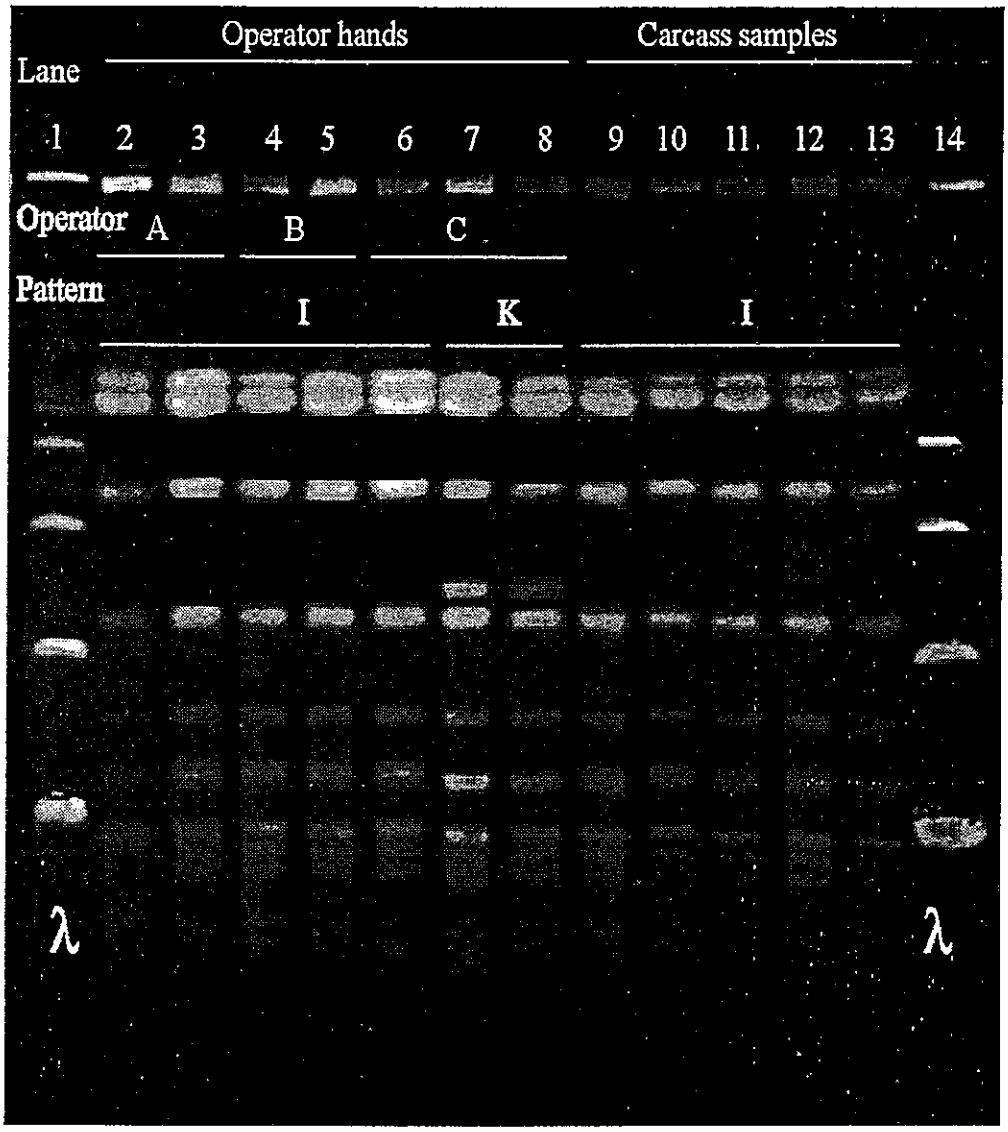


Figure 5: Pulsed field gel electrophoresis patterns of coagulase positive staphylococci isolated from the hands of evisceration workers (lanes 2-8) before and during work, and from carcasses (lanes 9-13) immediately after evisceration. All isolates produced staphylococcal enterotoxin. Lanes 1 and 14 contain the lambda molecular weight marker.