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Incidence and properties of *Staphylococcus aureus* associated with turkeys during processing and further-processing operations

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SUMMARY

The incidence of *Staphylococcus aureus* on turkeys sampled at various stages of processing and further-processing was determined on four occasions at each of three different processing plants. For freshly-slaughtered birds, counts from neck skin varied from plant to plant over the range $< 10^2$ to $> 10^5$/g but in all cases the corresponding counts obtained from carcasses sampled after chilling rarely exceeded 10^3/g and the same was true for samples of mechanically recovered meat (MRM), the final raw product examined.

Despite the limited susceptibility of isolates from the different factories to typing by means of either standard human or poultry bacteriophages (55–94 % untypable), evidence was obtained with the aid of biotyping for the presence of both human and animal-derived strains. However, some biotypes isolated from MRM were not detected at earlier stages of processing.

At one processing plant, an 'indigenous' type of *S. aureus* was clearly demonstrated. It occurred in high numbers in the defeathering machines (up to 10^5/swab), was found on carcasses at all subsequent stages of processing over the survey period and was shown to survive routine cleaning and disinfection procedures. Isolates of this type produced unusually large amounts of extracellular 'slime' in artificial culture.

Two of the three processing plants yielded isolates which were enterotoxigenic. Of 55 strains from Plant 1, 60 % produced enterotoxin C and all were of the 'indigenous' type. In the case of Plant 2, only two type D- and one type F-producing strain were found.

INTRODUCTION

Staphylococcus aureus occurs in the upper respiratory tract and among the surface microflora of normal, live poultry (Devriese, Devos & van Damme, 1975), chickens being colonized by this organism during the first few days of life (Thompson, Gibbs & Patterson, 1980). Levels increase to a maximum at about the seventh week of life which is close to the usual slaughter-age for broilers in the U.K. The incidence, distribution and properties of *S. aureus* encountered in chicken processing are well documented (Gibbs, Patterson & Harvey, 1978; Gibbs, Patterson & Thompson, 1978*a*, *b*) and it has been shown that some strains may

become established in plant machinery (Gibbs, Patterson & Thompson, 1978*b*; Notermans, Dufrenne & van Leeuwen, 1982). Enterotoxin production by strains isolated from broiler chickens was demonstrated by Harvey, Patterson & Gibbs (1982) who found strains of both human and poultry origin as well as some with intermediate properties.

By comparison with chickens, data on the staphylococcal flora of live turkeys is sparse, although Smith *et al.* (1961*a*, *b*) developed a bacteriophage-typing system for strains isolated from turkeys with synovitis and studied the transfer of strains among an experimentally divided flock.

In a study of poultry processing which included turkeys, Lahellec, Colin & Meurier (1977) showed that live bird strains of *S. aureus* tended to differ in certain physiological properties from those isolated from processed carcasses and attention was focussed on the defeathering machines as a major source of product contamination. However, no information was given on the enterotoxigenicity of the isolates obtained.

Recently, there has been a marked expansion in the U.K. in the development of further-processed convenience foods manufactured from turkey meat. Some of these products e.g. sausages are based on mechanically recovered meat (MRM) which is removed from the bones as the residue after manual deboning operations. The MRM is prone to contamination with organisms such as *S. aureus*, partly because of the handling which occurs at the previous stages.

The present study was undertaken to determine the effect of initial contamination and subsequent processing on the numbers and types of *S. aureus* associated with the final raw product, whether an oven-ready carcass or MRM. Data are also presented on the incidence of enterotoxin-producing strains among isolates from turkeys.

MATERIALS AND METHODS

Processing plants

Plants 1 and 2 were used exclusively for processing turkeys whereas at Plant 3 turkey-processing was usually preceded by a period during which chickens were processed. The change-over did not involve any special cleaning and disinfection of the equipment.

Plant 1

At this plant hens and stags aged 11–14 weeks were processed at a rate of 3500/h. After being water-scalded at 58 °C, the birds passed through a series of four defeathering machines. Then they were eviscerated manually, spray-washed and chilled in a series of six counterflow water-immersion chillers. All process water used was chlorinated to a level of 10–20 mg/l total residual chlorine. After holding overnight in tanks of chlorinated slush-ice, the carcasses were either frozen or passed into the further-processing area for deboning and product manufacture.

Plant 2

Here the rate of processing was *ca.* 1500 birds/h, using 16-week-old stags. The processing procedures were similar to those at Plant 1 except that only two

defeathering machines and two counterflow water chillers were used. There was no super-chlorination of the process water.

Plant 3

In this case the birds being processed were 14–18-week-old hens and stags and the rate of processing was *ca.* 1400 birds/h. The scald temperature was 52 °C. Again, processing procedures were similar to those at Plant 1 except that carcasses were air-chilled.

Sampling procedures

Each plant was visited on four separate occasions over a period of 6 months. During each visit, birds from one flock were sampled at both primary and further-processing stages.

Samples of neck skin (*ca.* 5 g) were taken aseptically after the following stages of processing: bleeding, defeathering, evisceration, chilling and overnight holding in slush-ice. In the further processing area the samples taken were (*a*) *ca.* 5 g of residual muscle from the frame after manual deboning (*b*) *ca.* 5 g of MRM taken from the outlet of the machine which was of the same type (Protecon U.K. Ltd, Bedford, England) at all three plants studied.

Water samples were taken from the mid-point of each chiller during processing of the test flock. The method used for this and the determination of total residual chlorine in process water was as described by Mead & Thomas (1973).

Swab samples from the defeathering machines were collected both during processing of the flock and on the following day after cleaning and disinfection of the equipment but before the start of processing. On each occasion, a complete rubber 'finger' was swabbed using two cotton wool swabs (Exogen Ltd, Glasgow, Scotland), moistened if necessary with diluent containing 0·1 % peptone (Straka & Stokes, 1957). After sampling, the swabs were transferred to 9 ml of the diluent.

Microbiological examination

All samples of skin and muscle were weighed and then macerated for 2 minutes in 45 ml of 0·1 % peptone diluent, using a Colworth Stomacher (Seward Laboratory Ltd, London, England).

Appropriate ten-fold dilutions of each type of sample were prepared and plated on Baird-Parker agar containing 50 μg/ml sulphamezathine (Smith & Baird-Parker, 1964). Following incubation at 37 °C for 48 h, plates were counted and representative colonies picked for further examination. Each strain was checked for purity and the ability to produce coagulase by a standard tube test, as described below. Only coagulase-positive strains were studied further.

Biochemical tests

Coagulase. The test involved the use of rabbit plasma (Wellcome Diagnostics Ltd, Dartford, England), which was diluted 1 : 6 with 0·9 % w/v saline. The method used was that recommended by the manufacturer.

Test cultures were prepared in Difco Heart Infusion Broth and incubated at 37 °C for 24 h.

Caseolysis. The medium used was a buffered caseinate agar as described by Martley *et al.* (1970) and Martley, Jayashankar & Lawrence (1970).

After incubation of the plates at 37 °C for 48 h the patterns of casein degradation were graded A–D.

Gelatinase. The method and medium described by Gibbs, Patterson & Harvey (1978) was used. A clear zone of at least 2 mm in width was recorded as positive.

DNA hydrolysis. This was determined by the method of Jeffries *et al.* (1957) using Difco Heart Infusion Agar containing 0·2 % DNA (Sigma Chemical Co., Ltd, Poole, England) and by the toluidine blue – DNA agar (TDA) microslide method of Lachica, Genigeorgis & Hoeprich (1971), using dialysis sac-culture supernatants as the test material.

Crystal violet agar test. The appearance of 'macro colonies' on nutrient agar containing 0·001 % crystal violet was determined by the method of Meyer (1967). After incubation of the plates at 37 °C for 24 h CV-positive colonies were orange to orange/blue in appearance; those scored as CV-negative were violet to blue.

Fibrinolysin production. The method was that of Christie & Wilson (1941). Nutrient agar plates containing 10 % v/v precipitated rabbit plasma were used and after incubation at 37 °C for 24 h a zone of 2 mm around the colony was recorded as positive.

Resistance to mercury ions. The test was used by Lacey (1980) to facilitate the separation of human and animal strains of *S. aureus*. It involved spotting fresh broth cultures onto plates of Difco Heart Infusion Agar containing 50 μg/ml mercuric chloride, not the mercurous salt as originally described (Lacey, pers. comm.)

After incubation of the plates at 37 °C for 24 h the appearance of good growth was recorded as positive.

Bacteriophage typing

The methods and media used were described by Blair & Williams (1961) and Parker (1972). The strains were typed with two sets of phages: the International Human Set, supplied by the Central Public Health Laboratory, London, England and the Poultry Set described by Gibbs, Patterson & Thompson (1978*a*) and supplied by Dr J. T. Patterson.

Strains not typable with the International Human Set at routine test dilution (RTD) were re-tested at 100 × RTD. The poultry phages were used at RTD only.

Enterotoxin production

The production of enterotoxin involved the sac culture method of Donnelly *et al.* (1967) while toxins were detected using the optimal sensitivity plate (OSP) method described by Robbins, Gould & Bergdoll (1974). Reference enterotoxins A, B and C were supplied by Professor M. Bergdoll. Enterotoxin F and its corresponding antiserum were provided by Dr S. Notermans (National Institute of Public Health, Bilthoven, the Netherlands) who also carried out determination of enterotoxins D and E. In this laboratory each batch of antiserum was tested against the reference toxins and the supernatants from dialysis sac cultures of the reference strains for enterotoxins A (NCTC 10652), B (NCTC 10654) and C (NCTC 10655).

Table 1. *Incidence of* S. aureus in *processing and further-processing operations: Plant* 1

Sampling day...	Mean $\log_{10}$ organisms/g and standard deviation			
	1	2	3	4
Birds after:				
bleeding	4·17±0·76 (10)*	<1·60 (0)*	<1·60 (0)*	<1·60 (0)*
defeathering	3·57±0·49 (10)	2·89±0·64 (10)	2·75±0·25 (10)	2·25±0·27 (8)
evisceration	2·79±0·17 (10)	1·95±0·25 (8)	2·16±0·04 (2)	1·75±0·07 (4)
chilling	1·94±0·13 (6)	1·84±0·23 (2)	<1·60 (0)	<1·60 (0)
holding in slush-ice	2·58±0·50 (10)	1·60±0·04 (3)	1·58±0·07 (2)	<1·60 (0)
deboning	2·19±0·17 (3)	1·64 (1)	1·76 (1)	1·95±0·14 (3)
Mechanically recovered meat	2·29±0·39 (4)	1·48±0·09 (3)	1·55 (1)	2·48±0·47 (8)

* Number of samples positive/10 tested in each case.

Table 2. *Incidence of* S. aureus *in processing and further-processing operations: Plant* 2

Sampling day...	Mean $\log_{10}$ organisms/g and standard deviation			
	1	2	3	4
Birds after:				
bleeding	4·34±0·77 (9)*	4·68±0·77 (10)*	4·71±0·68 (9)*	3·53±0·93 (10)*
defeathering	2·78±0·52 (10)	3·33±0·60 (9)	2·82±0·59 (10)	3·09±0·44 (10)
evisceration	2·40±0·41 (10)	2·00±0·42 (8)	2·49±0·63 (9)	2·50±0·25 (10)
chilling	2·41±0·30 (10)	2·19±0·51 (10)	2·28±0·41 (10)	2·68±0·26 (10)
holding in slush-ice	2·78±0·20 (10)	2·65±0·68 (9)	2·81±0·40 (10)	3·07±0·49 (10)
deboning	2·47±0·92 (3)	2·59±0·96 (3)	2·36±0·23 (3)	2·06±0·37 (4)
Mechanically recovered meat	2·14±0·30 (6)	2·26±0·37 (7)	2·16±0·21 (7)	2·02±0·30 (9)

* Number of samples positive/10 tested in each case.

Table 3. *Incidence of* S. aureus *in processing and further-processing operations: Plant* 3

Sampling day...	Mean $\log_{10}$ organisms/g and standard deviation			
	1	2	3	4
Birds after:				
bleeding	2·99 ± 0·64 (7)*	2·57 ± 0·26 (7)*	4·12 ± 0·48 (10)*	3·30 ± 0·94 (5)*
defeathering	< 1·60 (0)	2·60 ± 0·29 (9)	2·47 ± 0·41 (6)	2·94 ± 0·32 (8)
evisceration	1·74 ± 0·17 (3)	2·47 ± 0·35 (8)	2·14 ± 0·26 (8)	2·43 ± 0·15 (2)
chilling	1·79 ± 0·27 (7)	2·67 ± 0·79 (9)	2·66 ± 0·49 (8)	2·60 ± 0·27 (8)
deboning	1·90 ± 0·25 (4)	1·83 ± 0·20 (2)	2·13 ± 0·39 (4)	2·36 ± 0·37 (9)
Mechanically recovered meat	2·58 ± 0·29 (10)	2·32 ± 0·16 (9)	2·56 ± 0·27 (10)	2·66 ± 0·36 (10)

* Number of samples positive/10 tested in each case.

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RESULTS

Quantitative aspects

Counts of *S. aureus* from birds sampled at various stages in processing for each of the three processing plants are shown in Tables 1–3.

At Plant 1 the organism was detected on freshly-slaughtered birds on only one of the four occasions that samples were taken. However, during subsequent visits the consistent presence of *S. aureus* on the outside of the birds following defeathering strongly suggests that the defeathering machines were responsible for contaminating the product. Despite this problem, the counts of *S. aureus* obtained from positive carcasses and MRM at later stages of processing were usually low, often being close to the limit of detection.

A different situation occurred at Plant 2 when relatively high counts of *S. aureus* were obtained from the majority of freshly slaughtered birds on each visit to the plant. Thereafter, counts declined to *ca* 10^2/g and usually remained at this level throughout the rest of the process.

At Plant 3 again relatively high counts of *S. aureus* were obtained from many of the birds sampled immediately after slaughter but levels declined to *ca* 10^2/g at later stages of the process. However, unlike the other plants, virtually 100% of MRM samples yielded *S. aureus* in this case.

Although water immersion chilling was used at Plants 1 and 2, superchlorination of the process water was used only at Plant 1 and on each visit to this plant no *S. aureus* was detected in the chillers. By contrast, the organism was readily isolated from the chiller water at Plant 2, occurring at up to $5{\cdot}0 \times 10^2$/ml in the first unit of the two-unit system.

Since the defeathering machines have been incriminated previously as a source of *S. aureus* contamination, swab samples were taken from the rubber 'fingers' at all three processing plants. In all cases, *S. aureus* was isolated at levels of 10^2 to 10^5/swab when samples were taken during the processing period and at 10^2 to 10^3/swab immediately after routine cleaning and disinfection at Plants 1 and 2. At Plant 3 the organism was less readily detected at this stage, being found only once at *ca*. 10^2/swab.

Properties of the strains

In total, 583 strains of *S. aureus* taken from each processing stage at the three plants were subjected to bacteriophage typing and a series of biochemical tests which permitted the recognition of five distinct groups or biotypes together with one group in which strains showed variable properties and were less readily sub-divided (Table 4). None of the strains in any group showed resistance to mercury ions.

The incidence of biotypes at different stages of processing at each plant is shown in Tables 5–7. The five biotypes recognized do not correspond exactly with those described previously (Hájek & Maršálek, 1971, 1973; Gibbs, Patterson & Harvey, 1978) but nevertheless show some correlation with the results of phage typing (Table 8).

Data for Plant 1 indicate that strains found on freshly-slaughtered birds (biotype 1) were largely replaced by strains of biotype 2 during the defeathering

Table 4. *Differentiation of biotypes*

Biotypes...	1	2	3	4	5	Unclassified
Reactions on caseinate agar	A	B/C	D	A	B	A–C
DNAse	+	+	+	+	+	+/−
Gelatinase	+	+	−	±	±	+/−
Crystal violet test	+	−	−	−	+	+/−
Fibrinolysin	−	+	+	+	+	+/−

A–D, graded reactions (see text); +, positive reaction; −, negative; ±, weak.

Table 5. *Incidence of different biotypes at various stages of processing at Plant* 1

Processing stage tested	No. of strains tested	No. of strains/biotype					
		1	2	3	4	5	Unclassified
After							
bleeding	9	9	0	0	0	0	0
defeathering	36	3	33	0	0	0	0
evisceration	21	3	18	0	0	0	0
chilling	8	4	4	0	0	0	0
holding in slush-ice	13	2	11	0	0	0	0
hand-deboned carcasses	8	2	6	0	0	0	0
MRM	22	4	6	12	0	0	0
Swabs from defeathering machines	9	0	9	0	0	0	0

Table 6. *Incidence of different biotypes at various stages of processing at Plant* 2

Processing stage	No. of strains tested	No. of strains/biotype					
		1	2	3	4	5	Unclassified
After							
bleeding	38	35	0	0	0	0	3
defeathering	40	40	0	0	0	0	0
evisceration	37	33	0	0	0	0	4
chilling	40	40	0	0	0	0	0
holding in slush-ice	38	37	0	0	0	0	1
Hand-deboned carcasses	14	13	0	0	0	0	1
MRM	31	29	0	0	0	0	2
Swabs from defeathering machines	13	13	0	0	0	0	0
Chiller water	7	7	0	0	0	0	0

stage (Table 5). Some of the biotype 1 strains were susceptible to phage 224 of the poultry group B_2, one of the 'live bird' groups described by Gibbs, Patterson & Thompson (1978*a*) whereas the biotype 2 strains were not typable with either the 'human' or 'poultry' sets of phages. When cultured in the laboratory, strains of biotype 2 were unusually adherent due to the production of extracellular slime. Colonies picked from agar plates tended to form 'elastic' strands whilst growth in liquid media led to an adherent film over the surface of the glass in contact with the medium. This type of strain was isolated from the defeathering machines before and after cleaning and disinfection, which would indicate its probable source.

Table 7. *Incidence of different biotypes at various stages of processing at Plant* 3

Processing stage	No. of strains tested	No. of strains/biotype					
		1	2	3	4	5	Unclassified
After:							
bleeding	31	0	0	0	7	0	24
defeathering	28	0	0	0	0	0	28
evisceration	20	0	0	0	3	0	17
chilling	34	0	0	0	3	0	31
Hand-deboned carcasses	19	0	0	0	1	0	18
MRM	45	0	0	0	0	26	19
Swabs from defeathering machine	8	0	0	0	0	0	8

Table 8. *Relationship between results obtained by means of bacteriophage typing and biotyping*

Plant no.	No. of strains tested	No. of strains typable (biotype classification in parentheses)		
		'Human' phage set	'Poultry' phage set	Both sets
1	132	12 (3)	17 (1)	0
2	263	5 (unclassified)	6 (1)	4 (1)
3	188	23 (4)	26 (unclassified)	35 (mostly 5)

By contrast with the above, strains of biotype 3 were isolated on one occasion from MRM and found to be typable with phage 85 (Group III) of the 'human' set but only at 100 × RTD.

Results from Plant 2 (Table 6) show a predominance of biotype 1 strains which appear to have been introduced by the in-coming birds. Few of these strains were typable with either set of phages.

Strains isolated from Plant 3 were less easily grouped as specific biotypes due to the variability of their properties (Table 7). Biotype 4 strains, typable with phages 29 and 52 (Group I) of the 'human' set were isolated from freshly slaughtered birds but only on one occasion. Other strains, typable solely with the 'human' phages (Group III), were included in the 'unclassified' group following biotyping but did not relate to any particular stage of processing. However, strains typable only with the 'poultry' set (phages 221, 225 and 215 of Groups B and C) and also of unclassified biotype were isolated from carcasses after defeathering and at subsequent stages of processing.

Strains of biotype 5 are of particular interest because they were isolated from MRM on three separate occasions and were not detected at any previous processing stage. They were typable with phages 53 and 85 (Group III) of the 'human' set as well as phage 220 (Group B) of the 'poultry' set and may represent a strain indigenous to the further-processing area.

Isolates representative of each biotype and phage type were tested for their ability to produce enterotoxin, as shown in Table 9. All strains producing enterotoxin C from Plant 1 were of biotype 2. The one strain from Plant 2 which produced enterotoxin F was of 'human' phage type isolated after evisceration,

Table 9. *Production of enterotoxins by turkey-associated strains of* S. aureus

Plant no.	No. of strains tested	% positive and type ( )
1	55	60 (C)
2	41	4 (D)
		2 (F)
3	50	0

whilst the two strains producing enterotoxin D were isolated from birds held overnight in slush-ice.

DISCUSSION

Although the number of samples examined in each case was small and within-plant variation was relatively large, the results obtained here suggest differences between the three processing plants in both the numbers and types of *S. aureus* occurring on the birds being processed. In the case of Plant 3 this situation may have been complicated by cross-contamination *via* the equipment from chickens processed earlier in the day on the same lines. However, the levels and types of *S. aureus* on in-coming birds which tended to vary from plant to plant are likely to have been influenced by the husbandry conditions in each case. For example, carriage of the organism on the external surface of chickens is known to be affected by the oral administration of certain antibiotics which can remove susceptible *S. aureus* populations and lead to the proliferation of new, resistant strains (Devriese, 1980). Despite the observed variation in initial numbers, levels of *S. aureus* on chilled carcasses rarely exceeded 10^3/g at any of the plants and the same was true of samples examined at subsequent stages.

Clear evidence was obtained at Plant 1 for the establishment of an 'indigenous' strain in the defeathering machines and the fact that this organism was enterotoxigenic is a matter of some concern. Strains of *S. aureus* have been shown to attach readily to different surfaces (Notermans, Firstenberg-Eden & van Schothorst, 1979) and the production of extracellular slime by the 'indigenous' strain is likely to contribute to this process, rendering removal more difficult. Also, in relation to the non typability of this strain by the 'human' or 'poultry' phages, the production of slime may provide a physical barrier which prevents the adsorption of bacteriophage, as proposed for encapsulated strains by Wilkinson & Holmes (1979).

The differentiation of *S. aureus* into 'human' or 'poultry' types by means of biotyping, as attempted by Hájek & Maršálek (1971) and Gibbs, Patterson & Harvey (1978) presents some difficulty since many strains possess characteristics of both types. Strains of biotype 1, as described in this paper, seem closely related to those of biotype B ('poultry' biotype) described by Gibbs, Patterson & Harvey (1978). It seems likely that these strains were in fact of poultry origin because most were isolated from in-coming birds and showed some reactions with phages of the poultry set.

Biotypes 2, 3 and 4 showed characteristics resembling those of biotype A ('human' biotype) of Gibbs, Patterson & Harvey (1978) although none showed resistance to mercury ions by comparison with 47 % of human strains studied by

Lacey (1980). Of the 113 strains belonging to these biotypes, 35% were typable with the 'human' phages, indicating a probable human origin.

Strains of biotype 5 showed properties of both 'human' and 'poultry' types in addition to being typable with both phage sets. This biotype and biotype 3 (Plant 1) were isolated only from MRM, the former biotype being found on three of the four occasions that samples were taken. Either it was a type 'indigenous' to the further-processing area or it was present on a site of the birds other than the skin and therefore was not detected by skin sampling at the various stages of primary processing.

Although the development of the 'poultry' phage set was shown by Gibbs, Patterson & Thompson (1978) to give valuable epidemiological information on the distribution of *S. aureus* in chicken processing plants, it is clear from this study that the 'poultry' phages are of limited use with turkey strains. Hence, it would seem necessary to use specific phages isolated from turkey strains of *S. aureus* or phages which are adapted to such strains, as demonstrated by Shimizu (1977). This would permit a closer scrutiny of turkey processing which differs in some respects from that of chickens and would help in elucidating the significance of *S. aureus* contamination in further-processing facilities.

The occurrence of enterotoxigenic strains on healthy chickens has been reported by Shiozawa, Kato & Shimizu (1980) who found that 2% of their isolates produced enterotoxin C. Later, Harvey, Patterson & Gibbs (1982) showed that 23% of *S. aureus* isolates from chicken carcasses processed in N. Ireland formed enterotoxin D. In the present study, the high incidence of enterotoxin C – producing strains isolated from the birds at Plant 1 appears to be due to contamination from the defeathering machines which provide a warm, moist environment favouring microbial growth. From the hygiene standpoint, more attention needs to be given to the design of defeathering machines and to the search for a more suitable material to replace rubber for the plucking 'fingers' since at present these rapidly become worn and cracked and hence difficult to clean and disinfect properly (Gibbs, Patterson & Thompson, 1978).

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