

Plasmid Profiles as Indicators of the Source of Contamination of *Staphylococcus aureus* Endemic within Poultry Processing Plants

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A total of 530 strains of *Staphylococcus aureus* were isolated from the defeathering machinery of a chicken processing plant and from neck skin samples of carcasses at different stages of processing in two visits 4 weeks apart. Eleven different plasmid profiles were detected in the isolates, eight being common to both visits. The plasmid profiles of the strains forming the majority of the population on the freshly slaughtered birds were rarely present in the strains isolated from the pluckers (except at the entry to the first plucker) and were present in only a small proportion of the strains isolated from carcasses after plucking. However, the profiles from the strains isolated from the pluckers on both visits were different from those forming the majority of the population on the incoming birds but formed the major part of the carcass flora after plucking, suggesting that such strains were endemic. These strains were found as a small proportion of the isolates made from the incoming birds, suggesting that this was the route by which the endemic strains were introduced into the plant. Such endemic strains exhibited a clumping growth, even in liquid shake culture, which may have made it easier for them to become established on the pluckers and to resist cleaning and disinfection. This clumping phenotype was correlated with the presence of a 7.5-megadalton plasmid.

The establishment of *Staphylococcus aureus* on the defeathering machinery of poultry processing plants is a widely recognized problem within the industry (10). The presence of this indigenous flora may cause high levels of carcass contamination leading to rejection of meat for use in further processed products. Previous studies, using phage- and biotyping, have found the differentiation of endemic strains from those of the existing *S. aureus* skin flora of poultry to be difficult and unreliable (1, 14). Consequently the source of endemic strains has not been unequivocally determined.

In a previous study of *S. aureus* strains isolated from turkey processing plants (5), we demonstrated that strains identified by biotyping as endemic types could also be distinguished from coincidentally isolated live-bird flora by the use of plasmid profiles. In the present study, we have looked at the plasmid profiles of *S. aureus* isolates from chickens and a chicken processing plant in order to determine the source of the endemic strains. Related work, demonstrating an increase of *S. aureus* on carcasses after plucking and high counts of *S. aureus* within the pluckers themselves, suggested the presence of endemic strains on the pluckers of the processing plant examined in this study (C. E. R. Dodd, G. C. Mead, and W. M. Waites, submitted for publication).

MATERIALS AND METHODS

Strains. The strains were isolated in a chicken processing plant from carcass skin after skin maceration and from defeathering machinery by swabbing as described previously (1; Dodd et al., submitted). The defeathering machine consisted of a bank of three pluckers in sequence, and samples were taken from the entry and exit points of each plucker. From the isolates obtained for each skin sample during visits 3 and 4, 5 were selected to represent the range of colony types observed, and thus 50 strains were obtained from each carcass processing stage, viz., after bleeding, after scalding

(visit 4 only), after plucking, and after chilling. In addition, from the plucker samples obtained during visit 3, 5 isolates were selected from those isolated from each rubber finger, giving 10 strains at each plucker sampling point. From the plucker samples from visit 4, the number of isolates selected was doubled to 20 strains. Thus, in total, 210 strains were selected from visit 3 and 320 were selected from visit 4.

Screening for plasmid DNA. All strains were screened for plasmid DNA by the modified method of Birnboim and Doly (2) as described previously (5). Plasmid DNA was run on 0.8% Tris-acetate gel at 35 V for 16 to 18 h. Plasmid molecular weights were estimated by comparison with marker plasmids isolated from *Escherichia coli* reference strain V517 (9).

Production of penicillinase. Penicillinase production was detected by the method of Sng et al. (12).

Ability to grow in a clump. Strains were grown overnight at 37°C in tryptone soy broth (5 ml; Oxoid Ltd.) and were shaken at 230 rpm in a tube shaker (model G24; New Brunswick Scientific Co.). These were then examined for the production of visible clumps and adherence to the glass of the tube walls. Strains were scored as strongly clumping, weakly clumping, or nonclumping.

RESULTS

Plasmid profiles. The range of plasmid profiles obtained from the strains is shown in Fig. 1. As discussed previously (5), not all the bands detected are necessarily CCC DNA, but this is not critical for differentiation of isolates. Fifteen different profiles were evident in the strains from visit 3, and 16 were evident in the strains from visit 4, although some of these profiles have plasmids of related sizes. Most profiles showed relationships to those obtained previously from isolates of *S. aureus* made during turkey processing (5). Comparison of plasmid size suggested the presence of 10 profile types in the strains from visit 3 and 9 profile types in those from visit 4, 8 being common to both visits. These profiles, their frequencies, and their distribution through the

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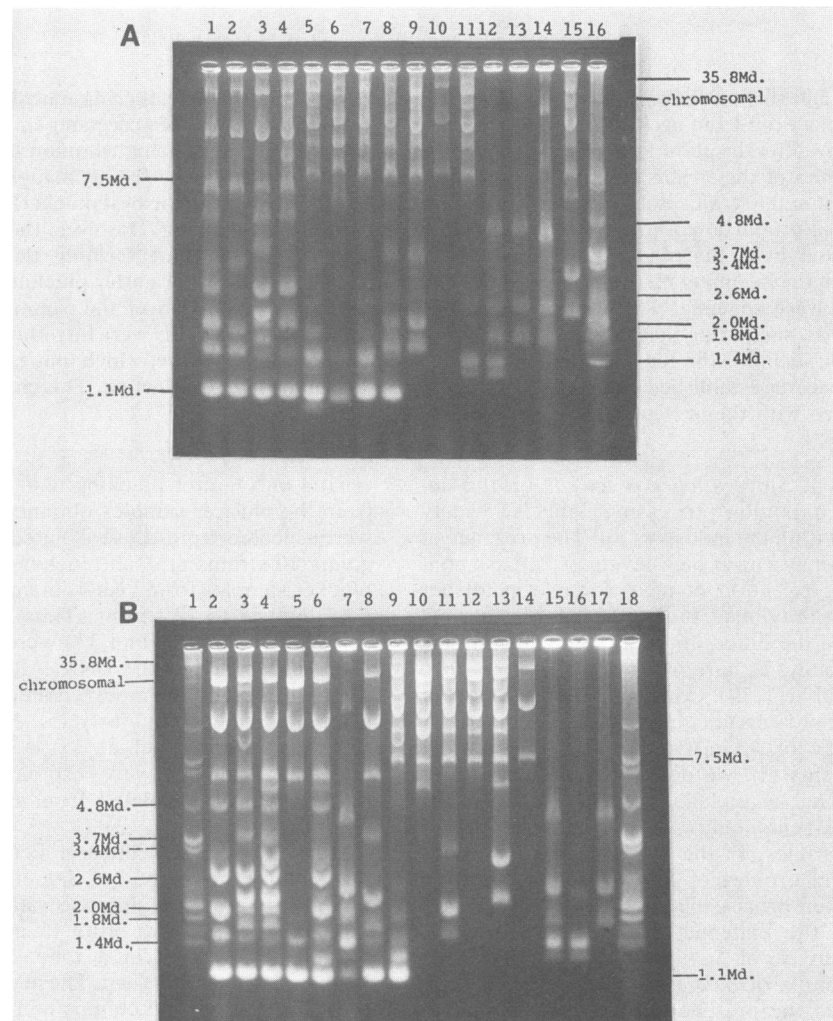


FIG. 1. Range of plasmid profiles from *S. aureus* isolates obtained during visit 3 (A) and visit 4 (B). (A) Lanes: 1 through 4, profile 1.1; 5 through 8, profile 1.1 + 7.5; 9, profile 1.4 + 7.5; 10, profile 7.5; 11, profile 1.2 + 7.5; 12, profile 1.2; 13, profile 1.2 (deleted); 14, profile 1.2 (deleted) + 7.5; 15, profile 2 + 7.5; 16 *E. coli* V517. (B) Lanes: 1, *E. coli* V517; 2 through 8, profile 1.1; 9, profile 1.1 + 7.5; 10, profile 6 + 7.5; 11, profile 1.4 + 7.5; 12, profile 7.5B; 13, profile 2 + 7.5; 14, profile 7.5A; 15 and 16, profile 1.2; 17, profile 1.2 (deleted); 18, *E. coli* V517. Profile 0 (no plasmid DNA) is not illustrated. Md., Megadalton.

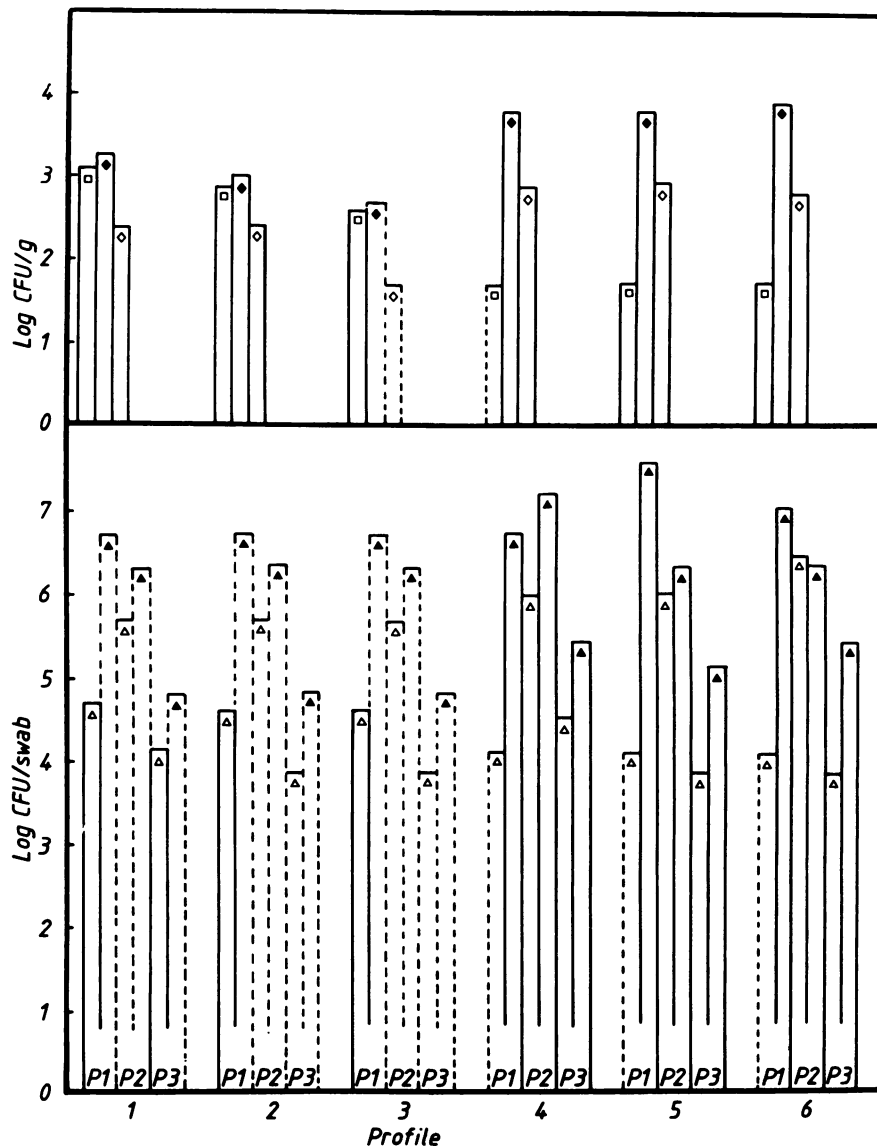


FIG. 2. Frequency and distribution of the six major plasmid profiles obtained on visit 3. Carcass processing stage symbols: □, after bleeding; ◆, after plucking; ◇, after chilling. P1, P2, and P3 represent pluckers 1, 2, and 3, respectively. Symbols: △, entry point; ▲, exit point. Plasmid profile: 1, 1.1; 2, 1.2 + 7.5; 3, 1.1 + 7.5; 4, 1.2; 5, 7.5A and 7.5B; 6, 0. Dotted lines indicate the calculated frequency of a profile at each stage based on that for a single strain and are shown where profiles were not found at a site to show their theoretical maximum levels in the population.

processing plant for each visit are shown in Tables 1 and 2, and data for the six major profiles from each visit are plotted diagrammatically in Fig. 2 and 3. The percent frequencies are based on 50 strains for each carcass processing stage, 10 strains for each plucker stage on visit 3, and 20 strains for each plucker stage on visit 4 (see Materials and Methods). Numerical frequencies were calculated from the percent frequency by multiplying by the mean count at that stage. Where a profile was not detected, its frequency in the population was less than that calculated for a single isolate, and this is indicated as less than (<) in Tables 1, 2, and 3 and as the theoretical maximum level by the dotted lines in Fig. 2, 3, 4, and 5. Strains containing a 1.1-megadalton (MDa) (1.6-kilobase-pair) plasmid showed much greater variety in the combination of plasmids they contained than the other profile types did.

In the strains from visit 3 (Table 1), there are six main profiles: 1.1, 1.2, 0, 7.5, 1.2 + 7.5, and 1.1 + 7.5. All except the 1.2 and the 1.1 + 7.5 profiles were found on the birds at all the stages of processing examined but in varying proportions.

Profiles 1.1, 1.2 + 7.5, and 1.1 + 7.5 were the major forms on the birds after bleeding, constituting 94% of the population, but only 16% of the population after chilling (with profile 1.1 + 7.5 not being found on the birds after the plucking stage). In contrast, profiles 0, 7.5, and 1.2 formed only 4% of the population on the birds before plucking (with profile 1.2 not found at all on the incoming birds) but 86% of the population after plucking and 78% after chilling. Moreover, there was a 60- to 75-fold increase in their numbers after the plucking stage. This would suggest that these latter profiles were associated with endemic strains, since there

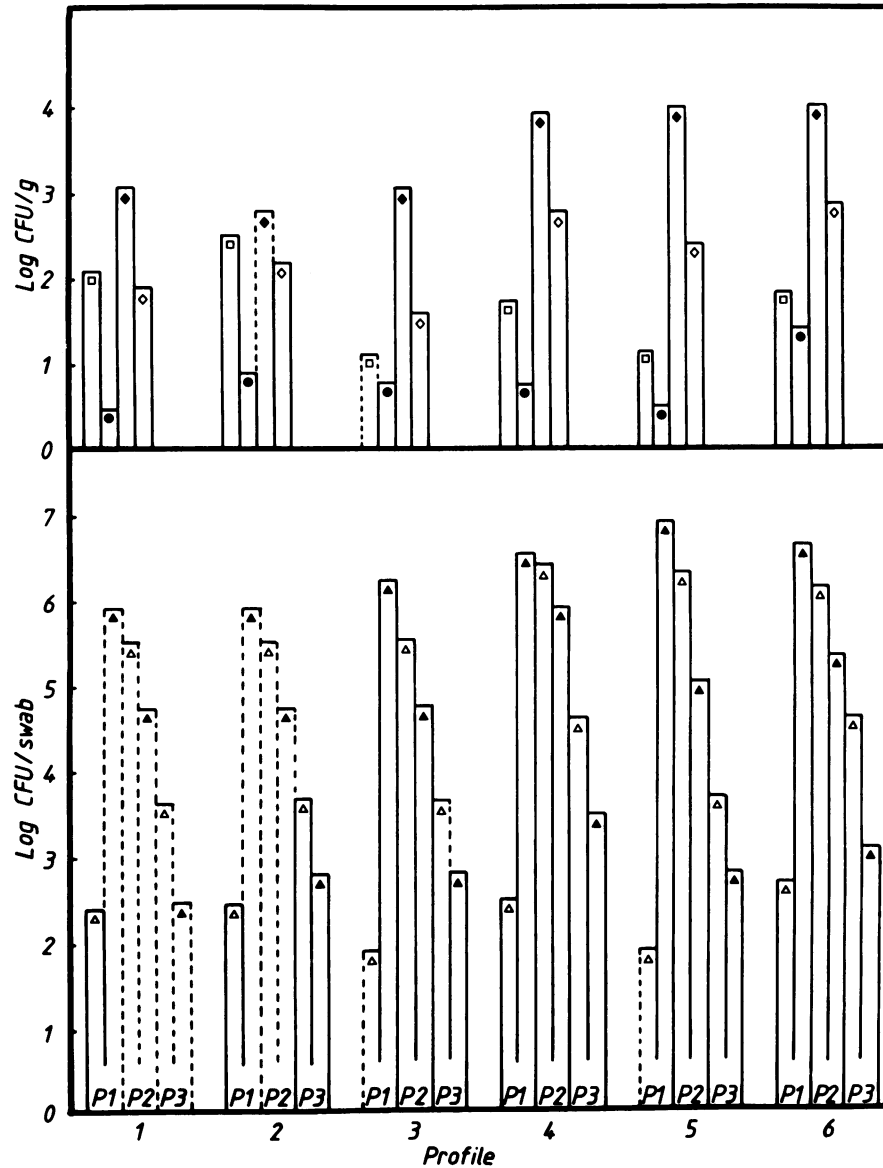


FIG. 3. Frequency and distribution of the six major plasmid profiles obtained on visit 4. Carcass processing stage symbol: ●, after scalding. Other symbols and abbreviations are described in the legend to Fig. 2. Plasmid profile: 1, 1.1; 2, 2 + 7.5; 3, 1.2 (deleted); 4, 1.2; 5, 7.5A; 6, 0.

was a significant increase in their numbers after plucking. Of the strains from the pluckers, only three profiles were evident at the entry to the first plucker, and these were the three main types found on the live birds. These profiles were rarely detected at other sampling points on the pluckers, and the profiles from strains isolated at the exit of plucker 1 were completely different. Only the three types found as the major constituents of the carcass population after plucking were found, that is, profiles 1.2, 0, and 7.5. These persisted throughout the rest of the pluckers, to the virtual exclusion of all other profiles. The appearance of strains with such profiles on the pluckers coincided with the large increase in numbers of *S. aureus*, which was apparently due to the presence of endemic strains (Dodd et al., submitted). It would therefore appear that strains with profiles 1.2, 0, and 7.5 were those endemic to the plant.

A similar situation was found on visit 4 (Table 2). There were six main profiles: 1.1, 1.2, 0, 7.5A, 1.2 (deleted), and 2 + 7.5, four of which were the same as the major profiles found on visit 3. There were two main profiles on the live birds, 1.1 and 2 + 7.5. Profile 1.1 was seen on visit 3 as one of the two major constituents of the live-bird flora, but the other major profile of visit 3 (1.1 + 7.5) was only a minor constituent of the flora on visit 4. Profiles 1.1 and 2 + 7.5 together were 72% of the population on the live birds but only 12% on the carcasses after chilling. A similar change was seen on visit 3. Profiles 1.2, 0, 7.5A, and 1.2 (deleted) formed 20% of the flora on the carcasses after bleeding but 96% after plucking and 76% after chilling. This result again suggested that these were the endemic profiles and was confirmed by their predominance at the exit of plucker 1 and throughout the rest of the pluckers. The two live-bird

TABLE 1. Distribution and frequency of plasmid profiles from visit 3^a

Plasmid profile	Frequency of plasmid profile in:																	
	Carcass processing stage						Plucker 1				Plucker 2				Plucker 3			
	After bleeding ^b		After plucking		After chilling		Entry		Exit		Entry		Exit		Entry		Exit	
	%	No. ^c	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
1.1	51	3.1	8	3.3	8	2.4	40	4.7	<10	<6.7	<10	<5.7	<10	<6.3	20	4.1	<10	<4.8
1.2	<2 ^d	<1.7	28	3.8	28	2.9	<10	<4.1	10	6.7	20	6.0	80	7.2	50	4.5	40	5.4
0	2	1.7	32	3.9	22	2.8	<10	<4.1	20	7.0	60	6.5	10	6.3	10	3.8	40	5.4
7.5 ^e	2	1.7	26	3.8	28	2.9	<10	<4.1	70	7.6	20	6.0	10	6.3	10	3.8	20	5.1
1.2 (deleted)	<2	<1.7	2	2.7	2	1.7	<10	<4.1	<10	<6.7	<10	<5.7	<10	<6.3	10	3.8	<10	<4.8
1.2 + 7.5	27	2.9	4	3.0	8	2.4	30	4.6	<10	<6.7	<10	<5.7	<10	<6.3	<10	<3.8	<10	<4.8
2 + 7.5	<2	<1.7	<2	<2.7	4	2.0	<10	<4.1	<10	<6.7	<10	<5.7	<10	<6.3	<10	<3.8	<10	<4.8
1.4 + 7.5	2	1.7	<2	<2.7	<2	<1.7	<10	<4.1	<10	<6.7	<10	<5.7	<10	<6.3	<10	<3.8	<10	<4.8
1.1 + 7.5	16	2.6	<2	<2.7	<2	<1.7	30	4.6	<10	<6.7	<10	<5.7	<10	<6.3	<10	<3.8	<10	<4.8

^a The mean counts (expressed as the log of the numbers per gram or swab) were as follows: after bleeding, 3.4; after plucking, 4.3; after chilling, 3.3; plucker 1, 5.0 (entry) and 7.7 (exit); plucker 2, 6.6 (entry) and 7.0 (exit); plucker 3, 4.8 (entry) and 5.7 (exit).
^b Numbers are based on 45 strains since 5 strains were excluded from these data (see Discussion).
^c Calculated as the percent frequency multiplied by the mean count.
^d Profile was not found, and occurrence was therefore below the frequency of a single strain at this stage (see Results).
^e This profile represents profiles 7.5A and 7.5B.

profiles (1.1 and 2 + 7.5) were found only at the entry to plucker 1, although profile 2 + 7.5 was present as a minor part of the plucker 3 flora.

The inclusion of samples after scalding (Table 2) showed that after plucking there was an increase in numbers not only of endemic types but also of the live-bird strains. The latter were not found in large numbers on the pluckers, which cannot therefore be the source of contamination.

Production of penicillinase. The ability of the strains to produce penicillinase was compared with the point of isolation in the processing plant (Table 3). Among the isolates from both visits, there was a very high proportion of strains on the incoming birds capable of penicillinase production and also a high proportion at the entry to plucker 1. However, there was a very low proportion of penicillinase producers on the rest of the pluckers, indicating that they were not establishing as endemic strains. This was reflected in the low proportion of penicillinase producers found on the

carcasses after plucking. The increase in the percentage of penicillinase producers on the chilled carcasses was not reflected in higher numbers of penicillinase producers and was therefore not a true increase in the level of contamination with these strains.

Ability to grow in a clump. A comparison of the abilities of the strains to grow as a clump in liquid culture and their points of isolation is shown in Table 3 and Fig. 4 and 5. The macroscopic appearance of the growth of the strains is shown in Fig. 6.

Few of the strains isolated from the birds after bleeding were clumping strains. However, after plucking there were significant numbers of strains showing this phenotype on the carcasses, and this correlated with the high numbers on the pluckers at the sites where the endemic strains were isolated (that is, the exit of plucker 1 and subsequently). Some weakly clumping strains were found at the entry to plucker 1, but strongly clumping strains were never detected at this

TABLE 2. Distribution and frequency of plasmid profiles from visit 4^a

Plasmid profile	Frequency of plasmid profile in:																			
	Carcass processing stage						Plucker 1				Plucker 2				Plucker 3					
	After bleeding		After scalding		After plucking		After chilling		Entry		Exit		Entry		Exit		Entry		Exit	
	%	No. ^b	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
1.1	20	2.1	6	0.5	4	3.1	4	1.9	15	2.4	<5	<5.9	<5	<5.5	<5	<4.7	<5	<3.7	<5	<2.5
1.2	8	1.7	10	0.7	28	3.9	28	2.7	20	2.5	20	6.5	40	6.4	65	5.9	45	4.6	50	3.5
0	10	1.8	46	1.4	32	4.0	34	2.8	30	2.7	25	6.6	20	6.1	20	5.3	45	4.6	20	3.1
7.5A ^c	2	1.1	6	0.5	32	4.0	12	2.4	<5	<1.9	45	6.9	35	6.3	10	5.0	5	3.7	10	2.8
7.5B ^c	4	1.4	4	0.3	<2	<2.8	8	2.2	15	2.4	<5	<5.9	<5	<5.5	<5	<4.7	<5	<3.7	<5	<2.5
1.2 (deleted)	<2 ^d	<1.1	12	0.8	4	3.1	2	1.6	<5	<1.9	10	6.2	5	5.5	5	4.7	<5	<3.7	10	2.8
2 + 7.5	52	2.5	14	0.9	<2	<2.8	8	2.2	20	2.5	<5	<5.9	<5	<5.5	<5	<4.7	5	3.7	10	2.8
1.4 + 2 + 7.5	<2	<1.1	2	0	<2	<2.8	<2	<1.6	<5	<1.9	<5	<5.9	<5	<5.5	<5	<4.7	<5	<3.7	<5	<2.5
1.1 + 7.5	2	1.1	<2	<0 ^e	<2	<2.8	4	1.9	<5	<1.9	<5	<5.9	<5	<5.5	<5	<4.7	<5	<3.7	<5	<2.5
6 + 7.5	2	1.1	<2	<0 ^e	<2	<2.8	<2	<1.6	<5	<1.9	<5	<5.9	<5	<5.5	<5	<4.7	<5	<3.7	<5	<2.5

^a The mean counts (expressed as the log of the numbers per gram or swab) were as follows: after bleeding, 2.7; after scalding, 1.8; after plucking, 4.5; after chilling, 3.2; plucker 1, 3.2 (entry) and 7.2 (exit); plucker 2, 6.8 (entry) and 6.0 (exit); plucker 3, 4.8 (entry) and 3.8 (exit).
^b Calculated as the percent frequency multiplied by the mean count.
^c Types A and B are defined in Results.
^d Profile was not found, and occurrence was therefore below the frequency of a single strain at this stage (see Results).
^e Less than 1 CFU/g.

TABLE 3. Distribution of penicillinase production and clumping phenotype

Point of isolation of strain	Percentage and no. of strains showing:											
	Penicillinase production				Ability to grow as a clump							
	Visit 3		Visit 4		Visit 3				Visit 4			
	%	No. ^a	%	No.	%		No. ^a		%		No.	
				Weak	Strong	Weak	Strong	Weak	Strong	Weak	Strong	
Carcass												
After bleeding	44	3.1	60	2.6	2	2	1.7	1.7	2	4	1.1	1.4
After scalding			20	1					2	14	0	0.8
After plucking	4	3.0	<2 ^b	<2.8	22	38	3.7	4.0	<2	36	<2.8	4.0
After chilling	12	2.5	20	2.6	20	34	2.7	3.0	<2	24	<1.6	2.7
Plucker 1												
Entry	60	4.9	35	2.7	<10	<10	<4.1	<4.1	5	<5	1.9	<1.9
Exit	<10	<6.7	<5	<5.9	<10	70	<6.7	7.6	15	60	6.4	7.0
Plucker 2												
Entry	20	6.0	<5	<5.5	30	20	6.1	6.0	20	35	6.1	6.4
Exit	10	6.3	<5	<4.7	30	50	6.8	7.0	45	25	5.7	5.4
Plucker 3												
Entry	<10	<3.8	5	3.7	40	40	4.4	4.4	65	10	4.8	3.9
Exit	<10	<4.8	10	2.8	50	40	5.5	5.4	10	25	2.8	3.2

^a Log of numbers per gram or swab, calculated from the percent frequency multiplied by the mean count (see Tables 1 and 2).
^b Characteristic was not found, and occurrence was therefore below the frequency of a single strain at this stage (see Results).

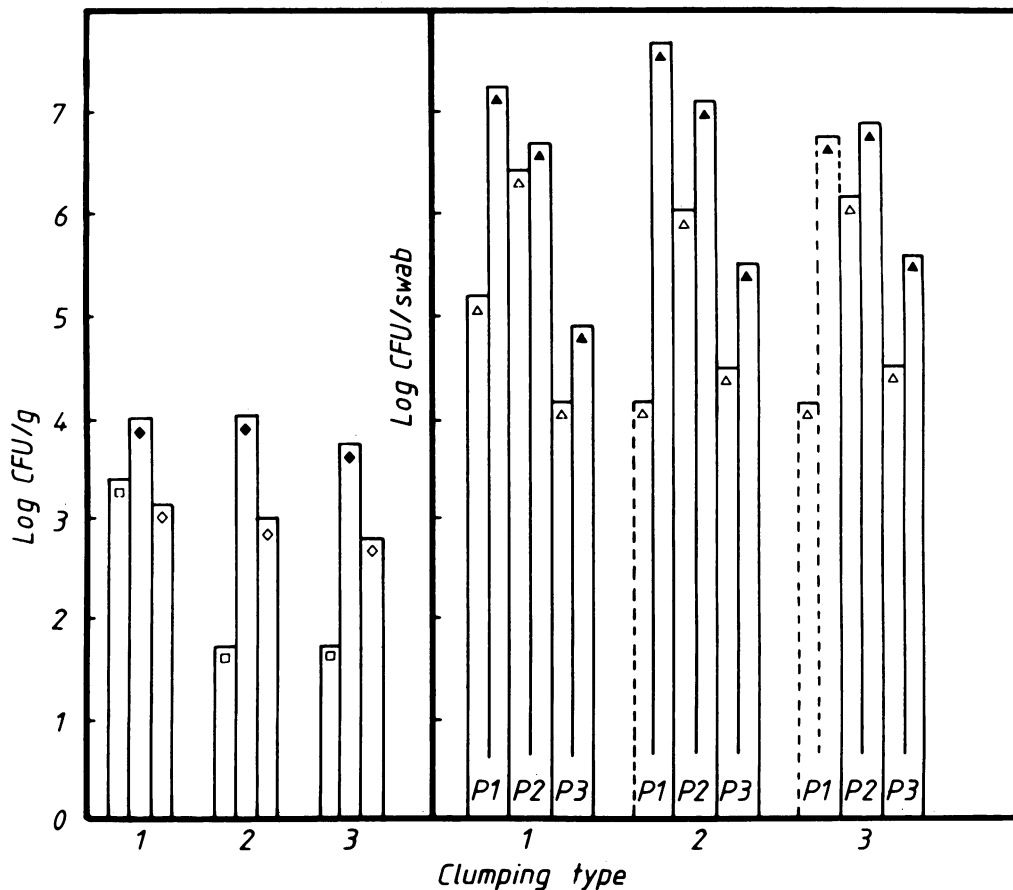


FIG. 4. Frequency and distribution of strains showing the clumping phenotype on visit 3. Symbols for processing stages are defined in the legend to Fig. 2. Clumping types: 1, nonclumping; 2, strongly clumping; 3, weakly clumping. Dotted lines indicate the frequency of a characteristic at each stage based on that for a single strain and are included where the characteristic was not found in order to show its theoretical maximum level in the population.

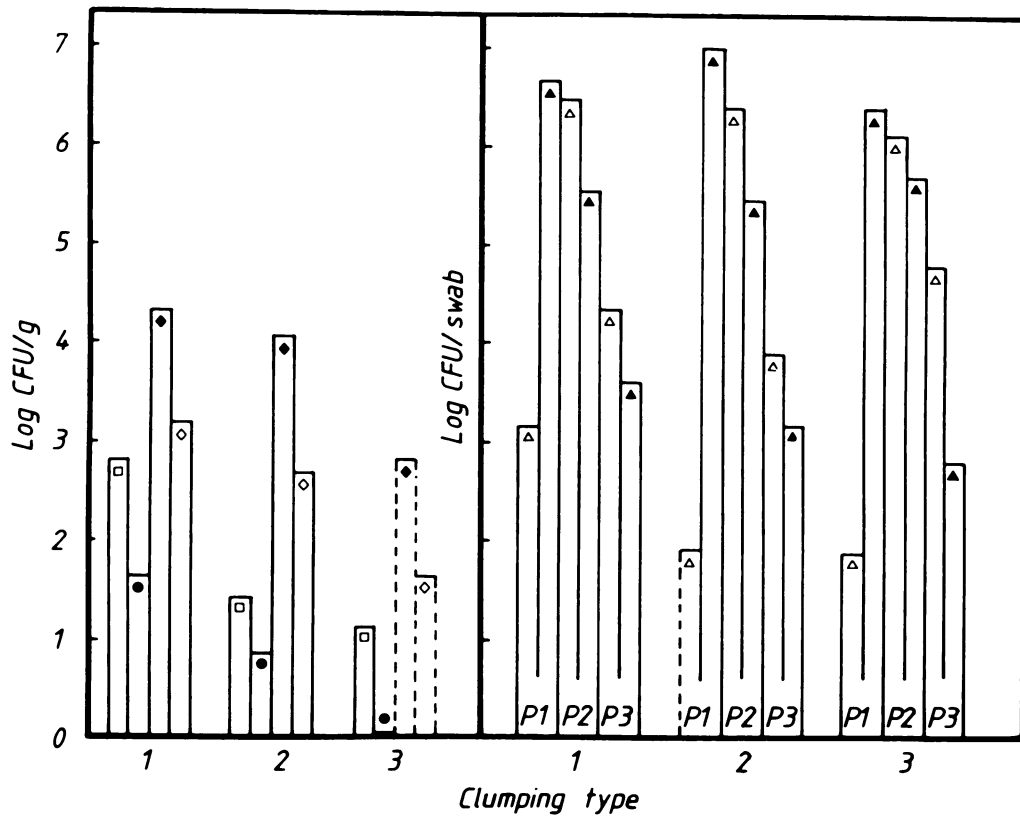


FIG. 5. Frequency and distribution of strains showing the clumping phenotype on visit 4. Symbols, numbers, and abbreviations are described in the legends to Fig. 2, 3, and 4.

site. There was a reduction in numbers of the clumping phenotype on the carcasses after scalding, although the proportion of clumping strains increased by 10%. Clumping strains may therefore be less easily removed or else more tolerant to the scald water. The numbers of this phenotype on the carcasses after chilling were also not as high as after

plucking, suggesting that these strains were removed by the washing action of the chillers and by superchlorination of the water.

Although strongly clumping strains were found as a high proportion of the strains at the exit of plucker 1 and thence in slightly lower proportions throughout pluckers 2 and 3 (although still as a substantial portion of the population), the proportion of the weakly clumping strains in the population increased on the rear pluckers. They also formed a smaller proportion of the population on the carcasses than the strongly clumping strains, suggesting that weakly clumping strains survive where the shear forces are less, that they transfer less efficiently to the carcasses, and that they are a lesser problem in carcass contamination.

Correlation of plasmid profiles with penicillinase production and clumping ability. In a previous study, we showed that the production of a penicillinase correlated closely with the presence of a 7.5-MDa (11-kilobase-pair) plasmid (5).

In the present study, of the 98 strains which produced penicillinase, 96 [98%] had a 7.5-MDa plasmid in their profile (Table 4). This again shows a strong correlation between penicillinase production and the presence of a plasmid of this size. A total of 183 strains had a plasmid of 7.5 MDa, but 88 did not produce penicillinase. However, all of these non-penicillinase producers were of the strongly clumping phenotype (Table 4), whereas none of the 96 penicillinase producers which contained a 7.5-MDa plasmid grew as a clump. Thus, there would appear to be two types of 7.5-MDa plasmid: type A, which is correlated with a strong ability to grow as a clump, and type B, which is correlated with penicillinase production. In the strains from visit 4 (Table 2),

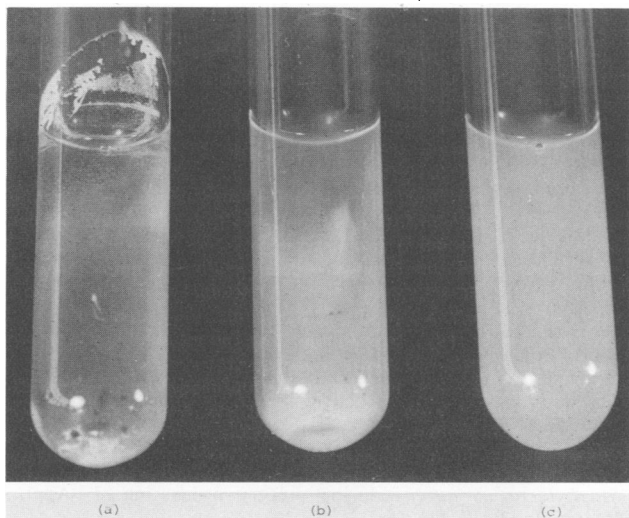


FIG. 6. Appearances of strains after growth in liquid shake culture: (a) strong ability to clump; (b) weak ability to clump; (c) inability to clump.

TABLE 4. Correlation of plasmid profiles with clumping ability and penicillinase production

Plasmid profile	No. of strains			Penicillinase production
	Total	Clumping ability		
		Weak	Strong	
1.1	57	1	0	0
2 + 7.5	46	0	0	46
6 + 7.5	1	0	0	1
1.1 + 7.5	13	0	0	13
1.4 (+ 2) + 7.5	2	0	0	2
1.2 + 7.5	21	0	0	21
1.2 (deleted) + 7.5 ^a	1	0	0	1
7.5	100	0	88	12
0	136	17	30	2
1.2	135	50	10	0
1.2 (deleted)	18	4	0	0

^a Profile deleted from Table 1; see Discussion.

only the profile with a 7.5-MDa plasmid alone was a mixture of types A and B (as shown); all other profiles containing a 7.5-MDa plasmid were of type B. These two 7.5-MDa types showed different distribution patterns in the plant (Table 2), as indicated previously, with the 7.5A type being endemic to the plant and the 7.5B type coming from the live-bird flora.

DISCUSSION

The source of *S. aureus* strains which become endemic within poultry processing plants has not been unequivocally determined, although it has been suggested that they are of human origin (3, 8). Thompson and Holding (13) suggested that endemic strains were not derived from poultry types. In a previous study (5), we showed that plasmid profiles could be used to distinguish endemic strains of *S. aureus* from those of the existing skin flora of turkeys. In this study, we have shown that this method can also be used for chickens. Moreover, the types of plasmid profiles found in the isolates are very similar. Certain profiles were found to be more prevalent on live birds, while others were more frequently associated with the defeathering machinery (that is, they were endemic). However, strains with endemic profiles were isolated from incoming birds, albeit as a very small proportion of the normal skin flora, and this appears to be the source from which endemic strains are introduced into the plant.

On both visits to the plant, the profiles associated with endemic strains were of the same types. This is predictable, as it is characteristic of endemic strains that they persist within a plant for some time, even though different strains were introduced on the live birds. On visit 3, one skin sample, taken from a bird after bleeding, became contaminated by contact with the floor in the bleeding area. Interestingly, all five isolates examined from this sample had one of the endemic profile types or a profile related to one of these, and the counts on this sample were equivalent to those obtained from the birds after plucking (these data were excluded from Table 1). This does suggest that other areas adjacent to the plucking machinery may become contaminated with endemic strains, probably via aerial contamination.

After plucking, the carcasses generally showed a small increase of strains which were not of the endemic types, that is, not detected in high numbers on the pluckers. Contami-

nation with these strains may come from the feathers. Thompson and Patterson (14) showed that *S. aureus* can be isolated in large numbers from feathers within the pluckers. Alternatively, contamination with these strains may result from their presence on the pluckers but at levels below the minimum detectable in this study (because of the extremely high numbers of endemic isolates). Such strains sometimes formed a detectable proportion of the population on the rear pluckers, where the overall counts were lower. However, their levels on the finished carcasses were never higher than those on the original birds, whereas the levels of endemic strains were always higher.

A high percentage of strains isolated from the incoming birds produced penicillinase, suggesting their prior treatment with penicillin. We found a similar result in another plant (5), confirming the results of other researchers (4, 6, 7). We have again demonstrated a close correlation between penicillinase production and the presence of a 7.5-MDa plasmid. On this occasion, we have also shown that the presence of a similarly sized plasmid is closely correlated with the ability of the strains to produce a strongly clumping phenotype during growth in liquid culture. Such an ability was associated primarily with endemic strains (defined by plasmid profiles) and may be an important factor in their colonization of the plucking machinery and in their transfer to and contamination of carcasses. Weakly clumping strains, although capable of colonizing the pluckers, appeared to be less important for carcass contamination. Clumping ability may also aid the strains to resist cleaning and disinfection, since Mead and Adams (11) have shown that they have a higher resistance to chlorine and Bolton et al. (2a) have demonstrated that single-cell preparations of a clumping strain were more sensitive to chlorine than cells growing in clumps. The cell surface features which induce the clumping phenotype have yet to be determined.

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