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ASPECTS OF THE MICROBIOLOGY OF CHICKEN CARCASSES

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INTRODUCTION AND OBJECTIVES

When assessing the microbial contamination on chicken carcasses after processing, it is important to know the areas most likely to be heavily contaminated. Since these areas are those likely to spoil more rapidly than less heavily contaminated areas the identification of those organisms developing during spoilage is of considerable interest. Little work appears to have been published dealing with the spread of contamination on chicken carcasses after processing. Ziegler, Spencer and Stadelman (1954) found the area under the wing to be the most heavily contaminated area of skin, and within the visceral cavity the area around the vent. This method of sampling was developed by Barnes and Shrimpton (1958) and Barnes (1960) who took 2g of skin from beneath the wing and 3g of surface tissue from around the vent, to give a total surface area of ca 50 cm^2.

Recent work in this laboratory has been concerned with finding the levels of bacterial contamination on 6 different sampling sites on the skin of cooled eviscerated, frozen eviscerated and uneviscerated (New York dressed) carcasses from 3 different processing plants. In an attempt to relate these findings to the flora developing in the skin and causing spoilage, several carcasses have been taken to spoilage and the aerobic microbial flora identified.

It has been suggested (Barnes and Impey, 1968) that certain isolates from spoiling poultry are sensitive to pH and these workers showed considerable differences between the pH values measured in different muscles of the chicken. When inoculated into minced breast (pH 5.7-5.9) and leg muscle (pH 6.4-6.7) pigmented and non-pigmented *Pseudomonas* grew well, but certain strains of *Acinetobacter* failed to grow in breast muscle, though showing growth in leg muscle. The growth of *Ps. putrefaciens* was more rapid in the leg muscle than in breast muscle. In light of these findings, pH measurements were also made on the muscle underlying the skin sampling sites, and on the skin of the neck.

METHODS

The carcases were obtained from 3 different processing plants. Plant A handled about 30,000 broiler chickens per day, all of which were eviscerated, cooled by water in spin-chiller tanks and frozen. An adjacent plant, B, produced about 5,000 cooled eviscerated and New York dressed (uneviscerated) carcasses the former being water-cooled in a spin-chiller, and the latter air-cooled. In-plant chlorination was practised throughout to about 20 p.p.m. free residual chlorine. Frozen and cooled eviscerated carcasses were also obtained from plant C which processed about 15,000 per day; the former were water-cooled in a spin-chiller and the latter air-cooled. In-plant chlorination was variable and not higher than 10 p.p.m.. The carcasses were sampled by using a sterile metal template to outline a circle of 10 cm^2 of skin at each sampling site and the enclosed skin was vigorously swabbed for 20 seconds with a sterile cotton-gauze swab (4cm x 15cm surgical cotton-gauze tightly wrapped around the end of a flat swabstick) and then the area of skin was removed using sterile scissors. The organisms were removed from the swab by shaking for 5 min in 10 ml 0.5 per cent peptone water using a laboratory flask-shaker and from the skin by shaking in a peptone-sand diluent, described below. The medium employed to cultivate the microorganisms from the skin was Oxoid Blood Agar Base, CM55, and replicate plates were incubated at 4° for 12 days, 15° for 6 days and 22° for 3 days. The sites sampled were: inside of drumstick, outside of drumstick, on the body wall under the wing, on the back, outside of the loose neck skin and on the body wall near the vent. Two further carcasses from plant C, one frozen eviscerated, the other cooled eviscerated and a frozen eviscerated carcass from plant A were stored at 2-4° in a laboratory

refrigerator until they were judged to be unusable due to off odours (8 days, 9 days and 14 days respectively). Areas of skin on 3 of the sites - near-vent, neck-skin and outside of drumstick - were removed and examined by shaking in peptone diluent with sand to act as an abrasive (Patterson, 1968). Twenty colonies for detailed identification were picked from suitable dilutions from the plates incubated at 4° and those incubated at 22°, to give a total of 120 isolates from each carcass. The pH of the underlying muscle was measured by inserting a glass electrode into the muscle, or in the case of the spoiled carcasses, on a macerated muscle sample in distilled water.

The following identification schemes were followed: Gram-negative rods, Hendrie, Hodgkiss and Shewan (1964); lactobacilli, Rogosa and Sharpe (1959); Microbacterium thermosphactum, Gardner (1966); Gram-positive cocci, Baird-Parker (1966).

RESULTS

The different levels of contamination and the spread of microorganisms over the skin is given in Table I, which shows the mean (swab + cut) values of the total counts obtained at 4°, 15° and 22°, since the interaction between sites and temperature was not significant. It can be seen that there are significant

Table I. Levels of microbial contamination at different sampling sites on chicken carcasses

Type of Carcass	Processing plant	Log_{10} nos. per cm^2 recovered from					
		Inside of drumstick	Outside of drumstick	Body wall, under-wing	Back	Neck skin	Near vent
Frozen eviscerated	A[1]	3.48	3.72	3.64	3.76	4.35	3.75
	C[2]	3.87	4.11	4.03	4.40	4.54	4.32
Cooled eviscerated	B[3]	3.69	3.94	3.78	4.07	4.47	3.98
	C[4]	3.17	3.06	3.04	3.57	4.24	3.05
Uneviscerated (New York Dressed)	B[5]	2.69	2.63	2.73	3.13	3.14	3.11

S.E. of a site mean = 0.0788 (55 d.f.)[1]; = 0.1257 (20 d.f.)[2];
= 0.1656 (20 d.f.)[3]; = 0.1198 (20 d.f.)[4]
= 0.1026 (20 d.f.)[5].

differences between certain sampling sites on carcasses from each processing plant. The neck-skin was highly significantly more contaminated than any of the other sites on the frozen eviscerated (plant A) carcasses, and significantly higher than the under-wing and inside of drumstick sites (plant B). With cooled eviscerated carcasses, the neck-skin was again significantly more contaminated than sites of lowest contamination (under-wing and inside of drumstick, plant A) and significantly higher than all other sites (plant C). On the uneviscerated, the neck-skin, back and near-vent sites were significantly more contaminated than the others but not different from

one another. The neck-skin receives considerable contamination during processing from the wash-water running off the carcass, and the back and near-vent sites can be soiled during evisceration.

The pH values obtained from the muscle underlying the skin at the various sampling sites (or of the loose neck-skin) are given in Table II. There are considerable differences between different sites, but in general the back, neck-skin and outside of drumstick gave the highest values, and the inside of drumstick, under-wing and breast sites the lowest, the differences being in many cases highly significant. The values obtained on the breast are very similar to those given by Barnes and Impey (1968), though the leg muscle results quoted by these workers (6.4-6.7) were higher. It is probable that these variations are due to the differences in position at which the readings were made.

Table II. pH of underlying muscle at different sampling sites

Type of Carcass	Processing plant	Inside of drumstick	Outside of drumstick	Body wall, under-wing	Back	Neck skin	Near vent	Breast
Frozen eviscerated	A[1]	5.97	6.34	5.88	6.29	6.30	6.14	6.06
	C[2]	5.84	6.29	6.15	6.43	6.40	6.30	5.84
Cooled eviscerated	B[3]	5.88	6.34	6.04	6.32	6.12	5.98	5.74
	C[4]	5.95	6.28	5.82	6.36	6.42	6.16	5.74
Uneviscerated	B[5]	6.18	6.20	5.90	6.28	6.28	6.04	-

S.E. of a mean = 0.084 (58 d.f.)[1]; = 0.152 (33 d.f.)[2]
= 0.057 (34 d.f.)[3]; = 0.098 (33 d.f.)[4]
= 0.084 (29 d.f.)[5]

The identification of the isolates from the 3 carcasses allowed to spoil is given in Table III, together with details of the total counts on the sites sampled and the pH of the underlying muscle. Each of the total count values had reached those indicated by Haines and Smith (1933) and Ayres (1960) for the appearance of off odours and slime on beef stored at 0°-20° i.e. $>10^7$ organisms per cm^2 and those quoted by Barnes and Shrimpton (1958)[18] spoiling chicken i.e. 7.5×10^7 for slight 'off' odour and 7.8×10^8 for strong 'off' odour. Colony counts at 20° given by these workers were 7.4×10^7 and 1.0×10^9 respectively. At the stage when spoilage was detected, 2 of the 3 carcasses had very high counts on the neck-skin (associated with high pH), suggesting that high initial counts (Table I) result in high counts after storage, with the probability of more rapid spoilage.

The identification of isolates (Table III) showed that those developing at 22° were mainly fluorescent and non-fluorescent pseudomonads with a smaller proportion of Ps. putrefaciens, and other Gram-negative rods (plant A), with large numbers of fluorescent pseudomonads near the vent. However, at plant C there was a higher proportion of isolates identified as Ps. putrefaciens and Lactobacillus spp. at all three sites. Differences were also obtained with the 4° isolates, those from plant A carcasses again being mainly fluorescent Pseudomonas spp. near the vent, and non-fluorescent on the neck-skin, whereas those from plant C again gave a higher proportion of Ps. putrefaciens. On both carcasses from this plant, the latter organisms constituted 40-50% of the flora on the 'near-vent' site. This organism was originally isolated from butter, and its properties have been described by Long and Hammer (1941). Ayres (1960) described its occurrence on 'slimy' chicken and on

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Table III. Identification of aerobic flora at different sites (as %) at the spoilage stage

Site	Count per cm^2	pH	No. of isolates	Gram-negative rods: Fluorescent pseudomonads	Gram-negative rods: Non-fluorescent pseudomonads	Gram-negative rods: Ps. putrefaciens	Gram-negative rods: Others: Aeromonas Flavobacterium Achromobacter etc	Gram-positive rods: Lactobacillus spp.	Gram-positive rods: Microbacterium thermosphactum	Gram-positive rods: Sporing bacilli	Gram-positive cocci: Coagulase-negative staphylococci	Yeasts	Unidentified
Frozen eviscerated carcass (Plant C)													
Near-vent	5.0×10^8 (22°)	6.8	20	10	25	40	10	5	5	0	5	0	0
	4.5×10^8 (4°)		20	20	20	40	5	0	10	0	0	5	0
Neck-skin	3.1×10^8 (22°)	6.9	20	10	25	30	5	25	0	0	0	0	0
	4.6×10^8 (4°)		20	15	25	20	5	25	5	0	5	0	0
Outside of drumstick	1.7×10^9 (22°)	6.8	20	15	25	30	5	25	0	0	0	0	0
	2.5×10^{10} (4°)		20	20	60	20	0	0	0	0	0	0	0
Cooled eviscerated carcass (Plant C)													
Near-vent	3.0×10^8 (22°)	6.3	20	0	45	40	0	10	0	0	0	0	5
	3.1×10^8 (4°)		20	0	35	50	15	0	0	0	0	0	0
Neck-skin	8.3×10^9 (22°)	7.5	20	0	65	20	0	0	0	10	0	0	5
	6.5×10^9 (4°)		20	15	65	15	5	0	0	0	0	0	0
Outside of drumstick	2.7×10^8 (22°)	6.45	20	10	75	10	5	0	0	0	0	0	0
	2.4×10^8 (4°)		20	15	75	10	0	0	0	0	0	0	0
Frozen eviscerated (Plant A)													
Near-vent	5.9×10^8 (22°)	6.55	20	55	40	5	0	0	0	0	0	0	0
	5.0×10^8 (4°)		20	50	40	5	0	0	0	0	0	0	5
Neck-skin	10.9×10^9 (22°)	7.35	20	10	65	5	20	0	0	0	0	0	0
	1.2×10^9 (4°)		20	5	90	5	0	0	0	0	0	0	0
Outside of drumstick	2.8×10^7 (22°)	6.7	20	20	10	20	15	5	25	0	0	0	5
	2.0×10^7 (4°)		20	40	25	10	5	5	15	0	0	0	0

sliced beef, and it has been found on spoiling poultry by Barnes and Impey (1968). The latter workers suggested that since the organism has lateral as well as polar flagella it should not be included in the genus Pseudomonas. Barnes and Thornley (1966) also found pigmented and non-pigmented strains of Pseudomonas predominating on chickens stored at 1° (71% of the flora) together with Ps. putrefaciens (19%) as the next major group. Ps. putrefaciens may be rather important from the spoilage point of view since it produces an obnoxious odour and appears to be extremely proteolytic. The reason for large numbers being present near the vent is not clear, but may be due to favourable conditions for growth due to soiling of this area during evisceration with blood and intestinal contents. Since both carcasses from plant C spoiled more rapidly than those from plant A, this may have been due to higher numbers of Ps. putrefaciens. Of 20 isolates studied by us in more detail, all showed extremely rapid reduction of litmus milk, rapid gelatin liquefaction, ability to hydrolyse casein, and produce H_2S in S.I.M. medium (Difco). The colony is typically pink to pink-brown, is oxidase positive, and gave a delayed positive catalase reaction. The isolates grew at 4° and 30° but not at 37°, with an optimum apparently at 22°. In this laboratory it has been noted frequently in large numbers, on nutrient agar plates on which samples were plated from chicken skin, water from spin-chillers, swabs from poultry plants, and even from poultry manure. The reason for large numbers on some carcasses at spoilage and not on others is not known.

Small numbers of other Gram-negative rods were identified as strains of Aeromonas, Flavobacterium, Achromobacter, and a lactose positive organism. The Gram-positive rods were lactobacilli resembling Lactobacillus plantarum, Microbacterium thermosphactum and two isolates of a sporing bacillus. Three isolates were classified as coagulase-ve staphylococci. Only one yeast was isolated, and 4 isolates could not be identified with certainty.

CONCLUSIONS AND PROJECTIONS

This work shows that the aerobic flora of the skin of chicken carcasses after processing is unevenly spread over the carcass and there are areas of higher contamination and other areas where the contamination is much lower. Areas of heavy contamination are those soiled during evisceration and handling, and in the case of the neck-skin by drainage from other parts of the carcass. The distribution and levels of contamination varies from plant to plant. Spoilage on the heavily contaminated sites is mainly by pigmented and non-pigmented Pseudomonas, with Ps. putrefaciens constituting a large proportion of the flora particularly near the vent. Other organisms present in fairly large numbers are Lactobacillus spp. and Microbacterium thermosphactum.

The work has not shown whether other sites would have a different spoilage flora, and which of the organisms were most active in spoilage. The effect of the pH of the underlying muscle and the pH of the skin on the flora developing on the skin needs further study. This work showed an increase in pH of the muscle and skin as 'off odours' developed. A study of the metabolic characteristics of the groups of organisms isolated, particularly as to their role in the spoilage of chicken skin and muscle would be valuable.

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