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Classification of Bacteria from Commercial Egg Washers and Washed and Unwashed Eggs

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A total of 432 bacterial isolates from washed and unwashed eggs, egg-washer surfaces, and washwaters from five egg-grading plants in Maryland and southeastern Pennsylvania were classified. Counts on equipment surfaces showed considerable variation from plant to plant, reflecting care used in cleaning. Unwashed eggs had a higher percentage of gram-positive cocci (71%), and isolates included *Streptococcus faecalis, Aerococcus,* and *Escherichia coli,* which were not isolated from equipment surfaces and washwaters. Equipment surfaces had a higher proportion of actinomycetes than unwashed eggs, and predominant gram-negative rods were *Alcaligenes* and *Moraxella,* which were not found on unwashed eggs. *Flavobacterium* and *Alcaligenes* have been implicated in shell egg rots, *Staphylococcus aureus* has been implicated in food poisoning, and organisms resembling micrococci and actinomycetes have been found in broken-out egg products.

Bacteria on egg shells have been implicated as a source of bacterial contamination of broken-out eggs (14, 21). Bacteria on shells may also, under certain conditions, penetrate through the shells into the interior and cause spoilage (4). Bacterial contamination of the shell surfaces by commercial egg washing is possible, and, in a previous study in our laboratory, I found a significant correlation between bacterial counts on shell surfaces and in washwater (16). Little attention has been given to the surfaces of egg-washing equipment such as brushes and conveyors as sources of contamination of washed eggs. However, Sayers (19) and Gillespie et al. (9) observed increased spoilage of eggs washed in equipment with surfaces that were heavily contaminated with bacteria.

There are only a few reports of the types of bacteria present on the shells of commercial hen's eggs (5, 10, 24). Mountney and Day (17) found a preponderance of gram-negative rods, mostly *Alcaligenes,* in washwater containing a quaternary ammonium sanitizer. Otherwise, there are no reports on types of bacteria present on washer surfaces or in washwaters.

For the present study, I have classified bacterial isolates from washwater, brushes, conveyors, and washed and unwashed eggs from five commercial egg-grading plants in Maryland and southeastern Pennsylvania.

MATERIALS AND METHODS

Sampling of eggs and washwater was done as previously described (16). Eggs were tested by two methods, a surface rinse and by evacuating and blending the shells. For the rinse method, each egg was immersed in 100 ml of tryptic soy broth (Difco) in a jar and shaken for 15 min on a mechanical rotary shaker. The rinsings from five eggs were combined. For the blending method, each egg was evacuated by suction through a small hole in one end, and the combined shells of five eggs were blended for 2 min in 200 ml of tryptic soy broth. Calcium alginate swabs were used for swabbing the brushes and conveyors, and each swab was dissolved in 100 ml of 0.5% sodium citrate–0.1% peptone. With the conveyors, the swab was pressed against the portion of the rubber roller that touched the eggs and was held in place as the roller turned. This was done five times for each swab. For the brushes, each swab was drawn along the outer surface of each brush for a distance of about 20 cm twice and pushed through the bristles twice. All swabs were taken in duplicate. Plate counts were made on tryptic soy agar (Difco) plates incubated for 4 days at 27°C.

For picking isolates, plates with well-separated individual colonies were used. Twenty colonies each were picked from plates from the washwater, conveyors, brushes, and washed and unwashed eggs (evacuation-blending method) for each plant, making a total of 500 isolates. Isolates were selected to be representative of types of colonies on the plate. Each pick was transferred to an agar slant containing 10 g of peptone (Difco) and 3 g of yeast extract per 1,000 ml. Each slant had 1 ml of sterile distilled water at the base. Slants were incubated at 27°C.

After visible growth appeared on the slants (usually 2 days), Gram stains were made, and the morphology of the organisms was determined. Motility was determined by microscopic examination of a drop of water from the base of the slant.

The isolates could be classified broadly into (i) gram-positive cocci (group 1), (ii) a variety of gram-positive or gram-variable asporogenous rods and irreg-

ular types (group 2), and (iii) gram-negative rods and cocci (group 3).

Group 1 (gram-positive cocci) were classified as described in Table 1. Aerobic and anaerobic utilization of glucose and mannitol were determined by using the media recommended by the I.C.S.B. Subcommittee (22). *Staphylococcus aureus* was differentiated from other staphylococci by anaerobic mannitol fermentation (2, 6). Streptococci were identified as *Streptococcus faecalis* by growth in 40% bile and 6.5% NaCl and fermentation of sorbitol but not arabinose. A few isolates were similar to micrococci except that they failed to produce a positive catalase test.

Group 2 (gram-positive or gram-variable rods) included mostly small asporogenous rods showing branching, V, or Y forms. Some showed a palisade arrangement. A few abnormally large cells (cystites) were present in a few cultures. A few also showed large rods arranged in loose chains. Many of these proved rather difficult to fit to known types of bacteria, but they seemed to belong to the general classification of actinomycetes. Some fitted descriptions of certain specific types and were classified as shown in Table 2. Others were classified as unidentified actinomycetes.

The gram-negative rods and cocci (group 3) were classified as in Table 3. *Enterobacteriaceae* were further classified as described by Edwards and Ewing (7).

TABLE 1. *Classification of gram-positive cocci*

Classification	Catalase	Action[a] on glucose	Morphology
Micrococcus	+	AO or N	Clusters or tetrads
Staphylococcus	+	AF	Clusters or tetrads
Aerococcus	−	AF	Clusters or tetrads
Streptococcus	−	AF	Chains

[a] AO, Acid oxidative; N, no change, AF, acid fermentative. See reference 22.

RESULTS AND DISCUSSION

The plate counts obtained from the samples of eggs, washwater, and equipment surfaces from which isolates were picked for this study are summarized in Table 4. Counts on equipment surfaces varied considerably from plant to plant, reflecting the care used in keeping the equipment clean. Counts on eggs were highly variable but were generally much lower on washed eggs, particularly by the surface rinse method. The high count obtained in plant 2 by the rinse method is apparently fortuitous since surface counts in other samples of washed eggs from this plant have been under 10^3 per shell. The picks of bacterial isolates of shell eggs were made from the plates from the evacuated and blended shells because only a few colonies were present on many of the plates prepared from surface rinses of the washed eggs.

The classification scheme used was generally based on the eighth edition of *Bergey's Manual* (6) with some guidance from other sources (1, 2, 7, 11, 23). Preliminary classification was based on Gram staining, morphology, action on glucose, catalase production, the oxidase test, and

TABLE 2. *Classification of gram-positive and gram-variable rods*

Classification	Action[a] on glucose	Motility	Catalase	Oxidase	Nitrate reduction	Other tests
Kurthia	K	+	+	−	−	
Microbacterium (small rods)	AF	−	+		−	
Arthrobacter	N	±	+		+	Gelatin liquefied
Propionibacterium	AF	−	−	−	+	Growth on 6.5% NaCl and 20% bile

[a] K, Alkaline; AF, acid fermentative; AO, acid oxidative; N, no change. See reference 11.

TABLE 3. *Classification of gram-negative rods*

Classification	Action[a] on glucose	Oxidase	Arginase[b]	Motility	Catalase
Enterobacteriaceae	AF	−		+ or −	+
Aeromonas (1)	AF	+	+	+	+
Vibrio (1)	AF	+	−	+	+
Pseudomonas	AO	+	+	+	+
Alcaligenes	N or K	+	−	+	+
Acinetobacter	N or K	−	−	−	+
Moraxella	N or K	+	−	−	+
Flavobacterium	N or AO; orange pigment	+	−	+ or −	+
Acetobacter	N or K; brown pigment			+	±

[a] K, Alkaline; N, no change; AF, acid fermentative; AO, acid oxidative. See reference 11.
[b] See reference 23.

motility. Some additional tests were performed as appeared necessary from the preliminary classification. There are numerous opportunities for misclassification in such a superficial examination of the isolates. However, with the large number of isolates, there was not enough time for a more detailed examination of each isolate. Many of the actinomycetes are variable in Gram staining and frequently coccoid at some stage of growth (6), and these, in particular, could be readily confused with other organisms. Further classification of the many isolates that had the general characteristics of actinomycetes was not attempted, although many biochemically resembled plant and animal pathogenic corynebacteria.

TABLE 4. *Log_{10} bacterial counts of equipment surfaces, washwater, and washed and unwashed eggs*[a]

Plant	Washwater (counts per ml)	Brushes (counts per swab)	Conveyors (counts per swab)	Eggshells[b] (counts per shell)			
				Unwashed		Washed	
				R	EB	R	EB
1	6.72	7.41	6.00	4.32	6.20	3.59	5.81
2	5.51	5.60	5.18	6.46	6.67	4.84	3.51
3	6.34	7.11	4.65	4.26	4.75	2.30	4.11
4	ND[c]	4.70	3.70	5.51	6.53	2.95	4.54
5	5.60	4.75	5.08	5.11	5.55	2.70	7.15

[a] Counts were made on tryptic soy agar incubated for 4 days at 27°C.
[b] R, Surface rinse method; EB, evacuation-blending method.
[c] ND, Not determined.

TABLE 5. *Percentages of types of microorganisms classified from isolates from five egg-grading plants*[a]

Microorganisms	Microorganism percentages in:				
	Washwater (73)[b]	Brushes (93)	Conveyors (77)	Eggs	
				Unwashed (93)	Washed (96)
Group 1 (gram-positive cocci)					
Total	59	20	52	71	43
Micrococcus	33	19	26	15	11
Staphylococcus					
S. aureus	3	—	4	3	7
Other	23	1	22	38	24
Aerococcus	—	—	—	8	—
Streptococcus faecalis	—	—	—	8	—
Group 2 (gram-positive and gram-variable rods)					
Actinomycetes					
Total	17	50	31	15	40
Arthrobacter	8	23	14	4	12
Kurthia	1	4	4	1	7
Propionibacterium	—	—	—	2	—
Microbacterium	—	—	—	—	1
Other (unidentified)	7	23	13	8	19
Bacillus	—	—	—	—	2
Lactobacillus	—	1	—	1	—
Group 3 (gram-negative rods and cocci)					
Alcaligenes	11	12	3	—	4
Moraxella	3	11	9	—	5
Acinetobacter	3	4	3	1	
Flavobacterium	4	2	3	1	1
Acetobacter	3	—	—	—	
Escherichia coli	—	—	—	10	3
Group 4 (yeasts)	—	—	—	1	1

[a] Isolates were from equipment surfaces, washed and unwashed eggs, and washwater. Because of rounding, some totals may not add up exactly.
[b] Parentheses indicate total number of isolates classified.

The classification of the isolates is summarized in Table 5. Data from the various sampling sites are combined. There was considerable variation (data not shown) among plants in types of bacteria isolated, but no pattern was obvious. No washwater sample was obtained from plant 4. The types of bacteria isolated from unwashed eggs differed notably from those isolated from equipment surfaces and washwaters. Gram-negative rods from unwashed eggs were mostly *Escherichia coli*, which were absent from equipment and washwaters. The proportion of gram-positive cocci was higher on the unwashed eggs and included types classified as *S. faecalis* and *Aerococcus*, which were not found in other samples. Equipment surfaces and washwater had a higher proportion of actinomycetes and considerable numbers of *Alcaligenes* and *Moraxella*, which were not found on unwashed eggs. The bacterial flora of washed eggs seemed intermediate between those of the equipment and the unwashed eggs, suggesting either contamination from equipment or selective survival of resistant types.

Bacteria found on equipment and in washwater would be expected to be types relatively resistant to the temperature (40 to 50°C) and alkaline pH (10 to 11) of the washwater. Kinner and Moats (J. A. Kinner and W. A. Moats, Poultry Sci., in press) found that most *Enterobacteriaceae* are killed rapidly under conditions like those in egg washers, so that their absence in the washer was expected. Staphylococci and *S. faecalis*, on the other hand, are relatively resistant to the alkaline pH conditions (Kinner and Moats, in press). The absence of *S. faecalis* on equipment is therefore somewhat surprising.

The types of bacteria found on shells of unwashed eggs in the present study are compared in Table 6 with those reported by other investigators (5, 10, 24). Since the eggs sampled in the present study were largely nest clean, only the results of other investigators with nest-clean eggs are included. Although there was considerable variation in the types found, gram-positive cocci always constituted a significant if not preponderant proportion of the isolates. Organisms of the coli-aerogenes groups were also consistently present. The occurrence of other types was highly variable.

Bacteria on shell surfaces have been found to be a major source of contamination of broken-out eggs (14, 21). Organisms resembling the micrococci and actinomycetes isolated in the present study were found to be pasteurization-resistant contaminants of egg melange from commercial breaking operations (18). These multiply rapidly to unacceptable levels in liquid egg products that are not properly refrigerated. Kraft et al. (13) reported that coryneform bacteria were among the most common contaminants of commercial liquid eggs. The surface rinse method used in this study is intended as a measure of surface organisms likely to contaminate the broken-out egg. Organisms that are not removed by a surface rinse are unlikely to contaminate the contents when the egg is broken out. Surface counts have generally been very low with commercially washed eggs, usually less than 10^3 per shell (16). Bacteria on the shell may also penetrate into the interior. Board (4) observed that regardless of the source of eggs or production practices, the types of organisms isolated from rotten eggs were similar and consisted of a small group of gram-negative rods. These organisms evidently have properties that favor growth inside intact shell eggs. Most organisms found on the shell surface are therefore harmless from the standpoint of spoilage of shell eggs. Of the organisms found in the present study, only *Alcaligenes* and *Flavobacterium* have been implicated in spoilage of shell eggs. However, Miller (15) isolated micrococci and *S. faecalis* from the contents of shell eggs, with *S. faecalis* present at up to 60×10^6 per g without, however, producing any detectable change in the egg. Fluorescent pseudomonads seem to be the most frequent cause of rots in shell eggs (3, 8, 12). None were isolated in the present study, and a systematic examination of plates for fluorescent colonies revealed only a few from one lot of

TABLE 6. *Percentages of types of microorganisms recovered from the shells of hen's eggs*

Microorganisms	Microorganism percentages in results of:			
	Haines (10) (nest run)	Zagaevsky and Lutikova (24) (nest clean)	Board et al. (5) (nest clean)	Present study (unwashed eggs)
Streptococcus		8		8
Staphylococcus	5	30	9	41
Micrococcus	18	23	37	15
Aerococcus				8
Sarcina	2	20		
Coryneform	3		5	15
Bacillus	30			
Lactobacillus				1
Pseudomonas	6		23	
Flavobacterium	3			1
Coli-aerogenes	5	19	11	10
Other aerobic gram-negative rods	24		15	1
Yeasts and molds	4			1

unwashed eggs. Actinomycetes are abundant in poultry litter (20), and *Kurthia* are reported to have been originally isolated from the intestinal contents of chickens (6), so the presence of these types on egg shells is not surprising.

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