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Airborne Microorganisms in Commercial Shell Egg Processing Facilities

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Abstract: Total aerobic bacteria, molds/yeasts, coliforms and pseudomonads in the air in three shell egg processing operations (in-line, off-line and mixed operations) were determined using MicroBio MB2 Air Samplers. Sites were sampled from each facility on three different days (replication) during the same week. Four air samples (1000 L each) were drawn from each sampling site on a given day. Sampling sites, included areas in or near the following on-site locations: hen house (in-line and mixed operations), farm transition room (in-line and mixed operations), egg washers, egg dryer, packer heads, post-processing cooler, nest-run cooler (off-line and mixed operations), loading dock and dry storage. Type of operation (in-line, off-line or mixed), sampling site and the interaction between operation and site had a significant effect on the number of total aerobic bacteria, molds/yeasts, coliforms and pseudomonads recovered ($P < 0.05$). Highest counts for total aerobic bacteria ($5.9 \log_{10}$ cfu/ml air), molds/yeasts ($4.0 \log_{10}$ cfu/ml air) and coliforms ($2.5 \log_{10}$ cfu/ml air) were found in the hen house. Highest counts for pseudomonads were found in the hen house ($3.2 \log_{10}$ cfu/ml air) and behind the egg washer ($3.5 \log_{10}$ cfu/ml air). Lowest counts for total aerobic bacteria ($2.5 \log_{10}$ cfu/ml air) and molds/yeast ($2.7 \log_{10}$ cfu/ml air) were found in the post-processing cooler. Few samples in the post-processing coolers, nest-run coolers, loading docks and dry storage areas tested positive for coliforms (0/36, 2/24, 1/36 and 0/36, respectively) and pseudomonads (1/36, 2/24, 5/36 and 6/36, respectively). Data gathered during this study has been useful in identifying the sources and levels of airborne contaminants in commercial shell egg processing facilities.

Key words: Shell eggs, airborne bacteria, bioaerosols, microorganisms

Introduction

Numerous studies have been conducted to evaluate the bacterial contamination of shell eggs during production and processing by sampling eggs, equipment, feed and the hens' reproduction tracts (Board *et al.*, 1964; Izat and Gardner, 1988; Baker, 1990; Barnhart *et al.*, 1991; Jones *et al.*, 1995; Bichler *et al.*, 1996; Hara-Kudo *et al.*, 2001; Knape *et al.*, 2002; Davies and Breslin, 2003; Jones *et al.*, 2003). For obvious reasons, the majority of these studies have focused on incidence or levels of *Salmonella*, although a few reports have addressed other pathogenic microorganisms and spoilage bacteria (Izat and Gardner, 1988; Baker *et al.*, 1987; Allen and Griffiths, 2001; Knape *et al.*, 2002). Jones *et al.* (1995) found *Salmonella* on 72% of the environmental samples collected from hen houses, 7.8% of the egg shells before washing and 1.1% of the egg shells after washing. These researchers did not find *Salmonella* in the contents of any of the eggs that they evaluated (Jones *et al.*, 1995). Knape *et al.* (2002) reported that the aerobic plate counts from egg rinses decreased by 2.9 and 1.5 \log_{10} cfu/ml for in-line and off-line eggs, respectively when counts on eggs at the transfer belt were compared to counts on eggs after washing. While these studies and others like them have provided critical information regarding direct product contamination, little attention has been given to the areas of indirect product

contamination. In 1964, Board *et al.* evaluated the microbiological contamination of egg shells and egg packaging materials and postulated that unpacking dirty eggs from flats created aerosols that could result in product contamination. However, no information is available regarding airborne contaminants and aerosol production during the processing of eggs. Moreover, egg processing facilities generally have limited physical barriers between operational stages; therefore, contaminated air could easily be circulated through the ventilation system, eventually coming into contact with finished product.

Airborne microbial populations and aerosol production has been examined in broiler houses, hatcheries, broiler processing facilities and turkey processing facilities. Lutgring *et al.* (1997) reported that counts of airborne bacteria (total aerobes) were highest in the shackling area of broiler processing facilities and decreased as the sampling progressed towards the product packaging area. These researchers and others noted that airborne populations were highest in the presence of the live birds because of wing flapping during shackling and potential movement and defecation during slaughter (Kotula and Kinner, 1964; Zottola *et al.*, 1970; Lenhart *et al.*, 1982; Lutgring *et al.*, 1997). Counts in the air ranged from 6.0 \log_{10} cfu/ml of air in the shackling room to 2.5 \log_{10} cfu/ml of air right

outside the facility (Lutgring *et al.*, 1997). In their survey of turkey processing facilities, Zottola *et al.* (1970) reported airborne *Salmonella* in 6 out of 7 facilities. *Campylobacter* has also been found in the air during shackling and defeathering of broilers (Whyte *et al.*, 2001).

Ellerbroek (1997) suggested that airborne bacteria may originate from contaminated surfaces or areas in the processing facility where cleaning and disinfection are minimal. It was also suggested that bacteria could become aerosolized when drains in a food processing facility are flooded during cleaning (Spurlock and Zottola, 1991). Jones *et al.* (2003) conducted a sanitation survey of commercial egg processing facilities and found no difference in bacterial counts for direct contact surfaces before and after routine sanitation. Based on the information from these previous studies, it is possible that bacteria on the equipment, employees, floors or drains could become aerosolized during operation and contaminate eggs, packaging material or other surfaces. In addition, egg processing facilities use a drying step immediately after washing where hot air is blown directly onto eggs. Although the majority of the eggs are clean at this stage, the egg dryer could be a source of airborne bacteria. The present study was performed to determine the microbiological contamination of air during the commercial processing of shell eggs.

Materials and Methods

Facilities and Sample Collection: During this study, air was sampled in three commercial shell egg processing facilities that were using different line operations (in-line, off-line and mixed operations). In an in-line operation, freshly laid eggs are transported from the hen houses to the processing facility via a series of conveyor belts that connect the hen houses to the processing facility. Eggs processed in an off-line operation originate from a location that is separate from the processing facility. A mixed operation is an in-line facility that also processes off-line eggs. For this study, each of the three types of line operations were visited on three separate days (replications) within the same week. For each replication, air samples were taken from 8 to 9 different locations throughout the facilities using four separate MicroBio MB2 Air Samplers (F. W. Parrett Limited, London, England). Prior to use, the air samplers were calibrated (certificate numbers 01211202 to 01211205) to a flow rate of 100 L/min using a standard vane anemometer (Airflow Developments AV-2, Buckinghamshire, England). All of the air samplers were placed onto standard camera stands using the placement screws on the stands and set to a height of 91.4 cm for sampling. This height was selected because it placed the sampling head at the approximated height of the eggs on the conveyor belts in the processing facility.

The inside and outside of each of the four sampling heads was sterilized with 70% ethanol and dried with clean Kimwipes (Kimberly-Clark, Roswell, GA) before sampling and between each sample. During sampling, the camera stands were aligned such that the sampling heads were within 15 cm of each other and faced in the same direction away from the sampling team. Air sampling sites, where applicable, included areas in or near the following locations: hen house (in-line and mixed operations), farm transition room (in-line and mixed operations), egg washers, egg dryer, packer head, post-processing cooler, nest-run cooler (off-line and mixed operations), loading dock and dry storage area.

Microbiological Analyses: At each sampling site, air was drawn for 10 min (1000 L of air) directly on to a Rodac plate (65 mm x 15 mm; Becton Dickinson Microbiology Systems, Sparks, MD) containing 5 ml of sterile Brain Heart Infusion (BHI) agar (Becton Dickinson, Sparks, MD). When the air sampling was near completion, lids for the plates were sterilized using 70% ethanol, dried with clean Kimwipes and put back onto the plate. These lids were then taped in place and the plates were put into sterile Whirl-PAK (Nasco, Atlanta, GA) bags and stored on ice for transportation to the laboratory. At the laboratory, BHI agar from each plate was aseptically removed using a new, sterile surgical blade (Feather Safety Razor Co., LTD. Japan) and placed into a sterile Whirl-PAK (Nasco, Atlanta, GA) bag with 10 ml of sterile phosphate buffered saline (PBS). These samples were blended for 2 min at normal speed (Seward Stomacher 80 Biomaster, Westbury, NY) and plated on the appropriate media. A preliminary study was conducted to determine the amount of BHI agar necessary to completely cover the bottom of the Rodac plates. During the preliminary study, 5 ml of BHI agar was found to be sufficient to collect air and allow for spiral plating the homogenized mixture of agar (with air sample) and PBS without clogging the stylus (Spiral Biotech Autoplate 4000, Norwood, MA).

Total aerobic bacteria in the homogenized mixture were enumerated using Plate Count Agar (Becton Dickinson, Sparks, MD). A 50 μ l aliquot of each air sample mixture was plated in duplicate using an automated spiral plater (Spiral Biotech Autoplate 4000, Norwood, MA) and the plates were incubated at 35 °C for 48 h. Molds and yeasts were enumerated by spiral plating 100 μ l of each air sample mixture onto duplicate plates of Potato Dextrose Agar (Becton Dickinson, Sparks, MD). These plates were incubated at 25 °C for 72 h. Coliform counts were determined by plating 1 ml from a serial dilution of the homogenized mixture onto duplicate *E. coli* Petrifilm™ (3 M Health Care, St. Paul, MN). After plating, Petrifilm was incubated at 35 °C for 48 h. Pseudomonads were enumerated by plating 100 μ l from

a serial dilution of the homogenized air sample mixture onto duplicate plates of Pseudomonas Base Agar (Oxoid, Ltd., Basingstoke, Hampshire, England). Pseudomonas plates were incubated at 25 °C for 48 h. Following incubation, colonies of total aerobic bacteria, molds/yeasts, coliforms and pseudomonads were counted and transformed into log₁₀ colony forming units (cfu) per ml of air. Volume of air included the initial 1000 L of sample drawn at each site along with the 5 ml of BHI agar, the 10 ml of PBS used to homogenize the agar and any additional dilution factors. Analyses were performed on the data after log transformation.

Statistical Analyses: Data were analyzed by the ANOVA option of the general linear model (GLM) procedure of SAS® using type of operation (in-line, off-line or mixed), sampling site and replication as the main effects (SAS, 1999). All first-order interactions were tested for statistical significance ($P < 0.05$) using the residual error mean squares. Data were pooled for all three replications and analyzed again after replication and the associated replication-interactions were found to be non-significant ($P > 0.05$). Means were separated using the least-squares means option of SAS® and reported along with the standard error (SAS, 1999). Microbial incidence was analyzed using the Fisher's Exact Test in the chi-square test for equal proportions.

Results and Discussion

Table 1 shows the effects of sampling site and type of commercial egg operation (in-line, off-line or mixed operation) on the log₁₀ cfu/ml air of total aerobic bacteria. Irrespective of the type of operation, highest counts for total aerobic bacteria were found in the hen house (5.5 to 5.9 log₁₀ cfu/ml air) and these values decreased as the eggs progressed to the post-processing (post-cooler) coolers (2.5 to 3.3 log₁₀ cfu/ml air). Counts in the hen house were similar to those previously reported for air in areas with animal confinement. Seedorf *et al.* (1998) reported total aerobic bacteria log₁₀ counts of 6.4, 5.1, 4.3 and 7.1 cfu/ml air for broiler houses, pig buildings, cattle buildings and hen houses, respectively. Moreover, these values are similar to the aerobic bacteria found on the farm belt (5.0 to 5.6 log₁₀ cfu/ml rinse) during a sanitation survey in commercial egg processing facilities (Jones *et al.*, 2003).

When counts found at the mixed operation were compared to counts at the in-line operation, greater numbers of total aerobic bacteria were found in the air in the farm transition (transition room) room (4.7 versus 3.7 log₁₀ cfu/ml air), post-cooler (3.3 versus 2.7 log₁₀ cfu/ml air), loading dock (3.8 versus 2.8 log₁₀ cfu/ml air) and dry storage (3.8 versus 3.1 log₁₀ cfu/ml air). While the counts taken in or near the hen house, egg washer and egg dryer were found to be statistically different among the different types of operations, the 0.5 log₁₀ cfu/ml

differences are not of biological or practical significance. Total aerobic bacteria counts were similar for the three different types of operations when the air samples were collected behind the packer heads (3.8 to 4.0 log₁₀ cfu/ml) and in the nest-run coolers (3.5 log₁₀ cfu/ml). The higher microbial counts in the mixed operation may be attributed to contamination from live birds in combination with eggs/carts/flats from different farms and additional product and employee movement through the facility.

Table 2 shows the effects of sampling site and type of commercial egg operations (in-line, off-line or mixed operations) on the log₁₀ cfu/ml air for molds and yeasts. Differences in molds/yeasts counts were minimal (< 0.5 log₁₀ cfu/ml air) among the types of operations when samples were collected in or near the hen houses, farm transition rooms, egg washers, egg dryers, packer heads, post-processing coolers and nest-run coolers. Molds/yeasts counts were 0.8 and 0.9 log₁₀ cfu/ml higher in the area near the loading dock for the mixed operation as compared to the off-line and in-line operations, respectively. In addition, there were higher molds/yeasts counts in the dry storage area of the mixed operation (3.8 log₁₀ cfu/ml) as compared to the in-line operation (3.1 log₁₀ cfu/ml). In the mixed operation, the loading dock and dry storage areas were in close proximity to one another, but were separated by a physical barrier. This was not the case for the in-line or off-line operations where the dry storage was in a different location in the facility.

Overall, the off-line operation had a higher incidence of airborne coliforms than the in-line and mixed operations, but the coliform numbers in the off-line operation were not always higher (Table 3). This was particularly noted behind the egg washers, egg dryer and packer head. All of the air samples collected behind the washer in the off-line operation tested positive for coliforms (12/12), but this sample was 0.4 log₁₀ units lower than the same sample collected in the in-line operation (1.8 log₁₀ cfu/ml versus 2.2 log₁₀ cfu/ml) where the prevalence was 5 out of 12. No difference was found in the coliform counts among the types of operations behind the egg dryer and packer head. Coliform counts were highest in air sampled in the hen houses, particularly for the in-line operation (2.5 log₁₀ cfu/ml) which was 0.8 log₁₀ units higher than the counts found in the hen house in the mixed operation (1.7 log₁₀ cfu/ml). Airborne coliforms were not detected in the post-processing cooler or the dry storage areas. Additionally, airborne coliforms were not detected in the nest-run cooler in the mixed operation or the loading docks of the in-line or mixed operations. The nest-run cooler and loading dock area of the off-line operation tested positive for airborne coliforms, but this was a low level of prevalence (2/12 and 1/12).

No difference was found in the airborne counts for pseudomonads in the hen house or farm transition

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Table 1: Effects of sampling site¹ and type of commercial egg operation (off-line, in-line or mixed) on log₁₀ counts (cfu/ml air) of airborne total aerobic bacteria

Sampling Site	Off-Line	In-Line	Mixed
Hen house	NP ²	5.5 ^b ± 0.03	5.9 ^a ± 0.20
Transition room	NP	3.7 ^b ± 0.10	4.7 ^a ± 0.03
Washers	4.3 ^a ± 0.10	4.2 ^a ± 0.22	3.7 ^b ± 0.06
Dryer	3.9 ^{ab} ± 0.08	3.5 ^b ± 0.07	4.0 ^a ± 0.07
Packer heads	3.9 ± 0.05	3.8 ± 0.13	4.0 ± 0.13
Post-cooler	2.5 ^b ± 0.11	2.7 ^b ± 0.08	3.3 ^a ± 0.06
Nest-run cooler	3.5 ± 0.10	NP	3.5 ± 0.19
Loading dock	3.8 ^a ± 0.33	2.8 ^b ± 0.15	3.8 ^a ± 0.19
Dry storage	3.6 ^a ± 0.18	3.1 ^b ± 0.14	3.8 ^a ± 0.09

^{a,b}Means ± standard error in the same row without common superscripts are significantly different (P < 0.05).

¹Prevalence (number of samples testing positive out of the total number of samples) for all sampling sites was 12/12 (4 samples per site for each of 3 replications). ²NP indicates that the sampling site was not present in the facility.

Table 2: Effects of sampling site¹ and type of commercial egg processing operation (off-line, in-line or mixed) on log₁₀ counts (cfu/ml air) of airborne molds and yeasts

Sampling Site	Off-Line	In-Line	Mixed
Hen house	NP ²	4.0 ± 0.23	3.9 ± 0.04
Transition room	NP	3.4 ^b ± 0.14	3.8 ^a ± 0.05
Washers	3.6 ± 0.09	3.8 ± 0.09	3.7 ± 0.10
Dryer	2.9 ^b ± 0.06	3.3 ^a ± 0.08	3.4 ^a ± 0.12
Packer heads	3.2 ± 0.10	3.3 ± 0.09	3.3 ± 0.05
Post-cooler	2.7 ^b ± 0.14	2.9 ^{ab} ± 0.07	3.1 ^a ± 0.16
Nest-run cooler	2.7 ± 0.11	NP	2.8 ± 0.15
Loading dock	2.8 ^b ± 0.15	2.7 ^b ± 0.08	3.6 ^a ± 0.14
Dry storage	3.6 ^a ± 0.04	3.1 ^b ± 0.10	3.8 ^a ± 0.05

^{a,b}Means ± standard error in the same row without common superscripts are significantly different (P < 0.05).

¹Prevalence (number of samples testing positive out of the total number of samples) for all sampling sites was 12/12 (4 samples per site for each of 3 replications). ²NP indicates that the sampling site was not present in the facility.

Table 3: Effects of sampling site and type of commercial egg processing operation (off-line, in-line or mixed) on the levels and prevalence of airborne coliforms

Sampling Site	Off-Line	In-Line	Mixed
Hen house	NP ¹	2.5 ^a ± 0.08 (12/12)	1.7 ^b ± 0.10 (12/12)
Transition room	NP	1.6 ± 0.39 (3/12)	2.0 ± 0.00 (1/12)
Washers	1.8 ^b ± 0.09 (12/12) ^x	2.2 ^a ± 0.08 (5/12) ^y	1.7 ^b ± 0.09 (2/12)
Dryer	1.0 ± 0.08 (7/12) ^x	NA (0/12) ^y	1.2 ± 0.00 (2/12) ^y
Packer heads	1.3 ± 0.10 (3/12) ^{xy}	1.7 ± 0.08 (4/12) ^x	NA (0/12) ^y
Post-cooler	NA (0/12)	NA (0/12)	NA (0/12)
Nest-run cooler	1.5 ± 0.37 (2/12)	NP	NA (0/12)
Loading dock	1.3 ± 0.00 (1/12)	NA (0/12)	NA (0/12)
Dry storage	NA (0/12)	NA (0/12)	NA (0/12)

^{a,b}Means ± standard error in the same row without common superscripts are significantly different (P < 0.05).

^{x-y}Prevalence (number of samples testing positive out of the total number of samples) in the same row without common superscripts are significantly different (P < 0.05). ¹NP indicates that the sampling site was not present in the facility, while NA indicates the absence of coliforms in the sample (incidence of 0 out of 12).

rooms for the in-line and mixed operations (Table 4). Counts for pseudomonads were highest in the hen house (3.1 log₁₀ cfu/ml), farm transition room (2.7 log₁₀ cfu/ml) and behind the egg washers (2.7 to 3.5 log₁₀ cfu/ml). The counts measured in the air behind the egg washers in the in-line operation were 0.8 log₁₀ cfu/ml

higher than those measured in the air behind the washers in the off-line operation. Only 1 out of 12 samples tested positive for pseudomonads in the air behind the washers in the mixed operation and this count (3.2 log₁₀ cfu/ml) was comparable to that found in the in-line operation (3.5 log₁₀ cfu/ml). Prevalence of

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Table 4: Effects of sampling site and type of commercial egg processing facility (off-line, in-line and mixed) on the level and prevalence of airborne pseudomonads

Sampling Site	Off-Line	In-Line	Mixed
Hen house	NP ¹	3.1 ± 0.25 (12/12) ^X	2.7 ± 0.10 (3/12) ^Y
Farm transition room	NP	2.3 ± 0.09 (6/12) ^X	2.2 ± 0.00 (1/12) ^Y
Washer	2.7 ^b ± 0.14 (10/12) ^X	3.5 ^a ± 0.34 (8/12) ^X	3.2 ^{ab} ± 0.00 (1/12) ^Y
Dryer	2.7 ± 0.15 (6/12) ^X	2.9 ± 0.15 (10/12) ^X	2.7 ± 0.00 (1/12) ^Y
Packer head	2.8 ± 0.59 (2/12)	2.3 ± 0.12 (5/12)	2.6 ± 0.09 (2/12)
Post-cooler	NA (0/12)	NA (0/12)	2.2 ± 0.00 (1/12)
Nest-run cooler	2.2 ± 0.00 (2/12)	NP	NA (0/12)
Loading dock	3.1 ± 0.00 (1/12) ^{XY}	NA (0/12) ^Y	2.8 ± 0.23 (4/12) ^X
Dry storage	2.2 ± 0.00 (1/12)	2.2 ± 0.00 (1/12)	2.5 ± 0.32 (4/12)

^{a, b}Means ± standard error in the same row without common superscripts are significantly different (P < 0.05).

^{X, Y}Prevalence (number of samples testing positive out of the total number of samples) in the same row without common superscripts are significantly different (P < 0.05). ¹NP indicates that the sampling site was not present in the facility, while NA indicates the absence of pseudomonads in the sample (incidence of 0 out of 12).

pseudomonads was low at all other sampling sites in the mixed operation. Counts for airborne pseudomonads measured behind the egg dryer, packer head, loading dock and dry storage were not significantly different among the different types of operations. Pseudomonad counts were lowest in the post-processing and nest-run coolers.

Data collected during this study demonstrate that airborne contamination varies among the different types of commercial egg processing operations. However for all commercial egg processing operations, airborne contamination of total aerobic bacteria, molds/yeasts, coliforms and pseudomonads was greatest in or near the live birds and lowest in the coolers (post-processing and nest-run). Commercial facilities could consider applying measures to reduce indoor contamination at the sites where the counts were the highest (hen house, farm transition room, behind the egg washers and egg dryer).

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