Research Notes

Enterobacteriaceae and related organisms isolated from nest run cart shelves in commercial shell egg processing facilities

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ABSTRACT Enterobacteriaceae, including Salmonella, may be recovered from foods and processing facilities. High levels of Enterobacteriaceae in the processing plant environment can be an indication of inadequate sanitation. This experiment was designed to determine if nest run egg carts serve as reservoirs for Enterobacteriaceae. Eggs that are produced by hens not housed in buildings connected to the processing plant are referred to as nest run. Many of these eggs are transported to the plant on carts to be processed. Two plants in the southeastern United States were sampled. On each of 3 visits, 5 shelves on each of 5 carts were sampled (n = 25/visit). A 12 × 12 cm area on each shelf was swabbed with a sterile gauze pad moistened with PBS and transported on ice back to the laboratory. Enterobacteriaceae were enumerated using violet red bile glucose agar incubated at 37°C for 24 h. There was 100% prevalence for Enterobacteriaceae at plant A with an average 3.8 log_{10} cfu/mL swab diluent. Plant B had 90% prevalence for Enterobacteriaceae with an average 3.2 log_{10} cfu/mL swab diluent. Two randomly selected isolates from each positive sample were recultured 3 times to increase the likelihood of clonality and were then identified biochemically. Of the 124 isolates analyzed, genera identified were Citrobacter spp., Escherichia spp., Enterobacter spp., Klebsiella spp., Hafnia spp., Kluyvera spp., Leclercia spp., and Salmonella spp. Pseudomonas spp. was the only non-Enterobacteriaceae identified by our methods. This work demonstrates that nest run egg carts serve as reservoirs for Enterobacteriaceae in the shell egg processing environment.

Key words: Enterobacteriaceae, shell egg, egg processing, sanitation, wood

INTRODUCTION

Sanitation is a crucial part of good manufacturing practices, ensuring that safe, wholesome food reaches consumers (Kornacki and Johnson, 2001). Clearly, surfaces of equipment, packaging, and other objects that come into direct contact with food are potential sources of recontamination. However, surfaces that do not come into direct contact must also be considered in terms of facility sanitation because of their potential to serve as reservoirs of microorganisms, which can decrease product safety as well as quality (Musgrove et al., 2004b).

In particular, equipment that travels throughout a facility may increase the risk of cross-contamination (Forsythe and Hayes, 2000). Commercial shell egg plants have unique challenges in maintaining sanitary conditions. In-line processors, in which the hen houses are physically connected to the plant, may have as many as 1 million birds essentially residing on the premises (Knape et al., 2002). Flies, dust, feces, and rodents are encountered more often than if the birds were housed elsewhere. A series of conveyers transports eggs from the cage or nest boxes to the washing, packing, and sorting equipment (Davies and Breslin, 2003). Processors who handle off-line eggs, produced by hens in houses not attached to the plant, may face even greater challenges. Off-line eggs are most often transported to the processing facility in plastic flats placed on metal carts with unpainted plywood shelves. Mixed operations will supplement their in-line egg production with off-line eggs, which are mechanically loaded onto the processing line from the plastic flats (Bell, 2002; Knape et al., 2002).

These nest run carts arrive at the shell egg facilities in trucks. Although great care is taken to maintain shell integrity, some eggs will be cracked during transport (Knape et al., 2002). Contents from broken eggs leak onto the unpainted wooden shelves, providing a nutritious substrate for bacteria and fungi. Shelving
is used indefinitely and cleaning them is not generally included in sanitation standard operating procedures. In a 5-yr cycle, many shell egg companies might post a profit for only 2 to 3 yr (Ricke et al., 2001). Operating on a thin profit margin, commercial enterprises avoid any unnecessary expense. Generally, the carts are only used for transport of prewashed eggs and eggs do not come into direct contact with the wood. As a result, alternative shelving for carts has not been considered a pressing issue (Bell, 2002).

After the carts have been used, they eventually become covered with dried egg yolk and albumen, providing a substrate for microbial growth, making a tempting potential microbiological sample. In a previous study, we found these shelves to be contaminated with large numbers of Enterobacteriaceae and aerobic microorganisms (Jones et al., 2003; Musgrove et al., 2004b). The objective of this study was to sample nest run cart shelves at 2 commercial shell egg processing facilities, an off-line (OL) and a mixed operations (MO) plant, respectively. Enterobacteriaceae populations were enumerated and a large number of presumptive Enterobacteriaceae isolates were identified to genus or species.

**MATERIALS AND METHODS**

**Sample Collection**

Two shell egg processing plants in the Southeast observed using nest run carts agreed to participate in the study. One of the plants was an OL facility. The other plant primarily processes in-line eggs but supplements production by processing nest run eggs (MO). Each plant was visited 3 times. At each visit, 5 nest run carts were randomly selected from a group of empty carts in the nest run cooler for sampling (n = 25/visit). These carts have metal frames with 3 pieces of unpainted plywood on each of 5 shelves. A 12 × 12 cm area on a single unpainted plywood section of each shelf was aseptically swabbed with a sterile gauze pad (10 × 10 cm) that had absorbed 10 mL of PBS, placed in a Whirl-Pak bag (Whirl-Pak, Modesto, CA), and transported on ice back to the laboratory within 2 h. The pad was squeezed before sampling the shelves so that excess diluent did not remain on the shelves. In the laboratory, samples were mixed by placement in a Pulsifier (Microbiology International, Frederick, MD) for 15 s.

**Microbiological Methodology**

Enterobacteriaceae were enumerated by pour-plating a 1-mL aliquot of each sample using violet red bile glucose agar (Accumedia Manufacturers Inc., East Lansing, MI). Each sample was plated in duplicate. An overlay of violet red bile glucose agar was poured after the original agar was set to facilitate the recovery of injured organisms (Hartman, 1979). Plates were incubated overnight at 37°C for 24 h. Plates with dark purple colonies with halos of bile salt deposition were considered positive and were counted. Counts were converted to base-10 logarithm colony-forming units per milliliter of rinse diluent.

**Selection of Isolates**

Two isolates from each positive sample were randomly selected using a numbered grid and a random number table (Steel and Torrie, 1980). Selected isolates were recultured 3 times onto plate count agar (Accumedia Manufacturers Inc.) and were incubated at 37°C overnight 3 times to increase the likelihood of clonality. After the third passage, samples were placed on ceramic beads in cryoprotective media and stored frozen at −80°C until further analyses were performed.

**Isolate Identification**

Frozen isolates were revived by placing beads onto plate count agar and incubating overnight at 37°C. Each isolate was tested for reaction to oxidase and results were recorded. A miniaturized strip system (bioMerieux Inc., Hazelwood, MO), reagents, and a database were used to identify each isolate biochemically. Isolates identified as *Salmonella* were confirmed using polyclonal antisera (Microgen, Camberly, UK).

**Statistical Analyses**

To analyze bacterial levels, SAS was used and χ² analyses were used to compare prevalence data (SAS Institute, 1994). Comparisons were made from plant to plant. A comparison of numbers of bacteria recovered from upper and lower shelves was conducted for each plant, respectively.

**RESULTS**

Levels and prevalence of Enterobacteriaceae were similar between the MO and OL plants. The average base-10 logarithm colony-forming units per milliliter of rinse diluent were 3.8 and 3.2 for MO and OL, respectively, whereas prevalence was 100 and 90%. The highest shelves were significantly less contaminated than the lowest shelves (P < 0.05; Figure 1) for the OL but not the MO plant. Isolate identifications are listed in Table 1. Enterobacteriaceae recovered from both plants included *Citrobacter diversus/amalonaticus*, *Citrobacter freundii*, *Enterobacter amnigenus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella ornthinolyltyica*, *Klebsiella oxytoca*, *Leclercia adecarboxylylata*, and *Salmonella* spp. *Enterobacter intermedius*, *Enterobacter agglomerans*, *Pseudomonas cepacia*, and *Kluyvera* spp. were recovered only from the MO plant, whereas *Enterobacter gergoviae*, *Enterobacter sakazakii*, *Escherichia fergusonii*, *Es-
Table 1. Isolate identification of Enterobacteriaceae and Pseudomonas cepacia recovered from nest run cart shelves in a mixed operation (MO) and off-line commercial (OL) shell egg processing facility.

<table>
<thead>
<tr>
<th>Genus/species</th>
<th>MO</th>
<th>OL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrobacter diversus/amalonaticus</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Enterobacter amnigenus</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>11</td>
<td>19</td>
<td>30</td>
</tr>
<tr>
<td>Enterobacter gergoviae</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter intermedius</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter sakazakii</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Escherichia fergusonis</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Escherichia vulneris</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Hafnia alvei</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Kluvyera spp.</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Leclercia adecarboxylata</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Pseudomonas cepacia</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>75</td>
<td>124</td>
</tr>
</tbody>
</table>

1Isolates recovered from swabs of nest run cart shelves by plating onto violet red bile glucose agar. After isolates were restreaked for clonality, biochemical tests were performed using API strips (BioMerieux, Hazelwood, MO) and posting results in the appropriate database.

2Number of isolates for a particular genus recovered from the MO, OL, or a total from both facilities.

Escherichia vulneris, and Hafnia alvei were recovered only at the OL plant. There were 49 isolates identified from the MO and 75 isolates identified from the OL plant.

**DISCUSSION**

In the last 100 yr, egg production in the United States has shifted from small free-range flocks kept to use wasted feed to highly mechanized operations physically connected to a series of houses, each housing 80,000 to 100,000 birds. Some in-line locations may have as many as 1,000,000 birds. Regulations are in place to ensure that consumers consistently receive high-quality shell eggs (Bell, 2002). Attention to details that preserve quality may have a beneficial effect on product safety; however, safety considerations are best managed by efforts, by industry, and regulators, which focus on safety. Many of the practices in the commercial industry, particularly in regards to sanitation, are based on history and economy more than sanitation or safety concerns (Bell, 2002). Eggs are not only highly nutritious, they are also much less perishable than poultry, meat, or dairy products; therefore, a less stringent level of sanitation may be observed without affecting safety or quality. However, now that food safety is becoming a regulatory focus for the shell egg industry, practices that were previously acceptable are now being reviewed (Jones et al., 2003; Musgrove et al., 2004b).

Minimum facility requirements are described in part 56 of 7 CFR, the regulations that govern the voluntary grading of shell eggs (USDA, 2000). Factors listed include the following: wooden benches in areas subjected to moisture and that develop odors shall be replaced with equipment of metal construction, surfaces that develop off-odors shall be replaced with materials that are impervious to moisture, and all egg contact surfaces are to be cleaned daily (USDA, 2000).

Wood shelves are inexpensive and have been used consistently for some time despite the fact that this material does not allow for easy cleaning (Forsythe and Hayes, 2000). Unlike other industries, the shell egg industry often operates on a thin profit margin; therefore, any changes made to equipment or sanitation practices must justify the expense. A more in-depth investiga-

**Figure 1.** Graph of average Enterobacteriaceae base-10 logarithm colony-forming units per milliliter recovered from shelves of nest run carts in mixed operation (MO) and off-line (OL) commercial shell egg processing facilities. Counts were determined by plating aliquots of shelf swabs onto violet red bile glucose agar incubated at 37°C overnight. Shelves for every cart sampled (5 carts/plant per rep) were numbered 1 to 5; the top shelf was labeled 1 and the bottom-most shelf was labeled 5. Bars for each plant (MO or OL) with different letters were significantly different. There were no differences between plants.
tion of nest run cart shelves was performed because in previous work performed in our laboratory, high microbial counts were detected for nest run cart shelves and wheels (Jones et al., 2003; Musgrove et al., 2004b).

Enterobacteriaceae counts may indicate poor sanitation. Coliform populations are used more often in the United States for the same purpose; however, this group of organisms, which is based on the ability to ferment lactose, is not taxonomically organized and excludes Salmonella and other lactose-negative microorganisms (Kornacki and Johnson, 2001). Enterobacteriaceae such as Proteus, Providencia, Shigella, Yersinia, as well as some species of Citrobacter, Enterobacter, Erwinia, and Serratia, are found on shell eggs and the shell egg processing environment (Board, 1966; Musgrove et al., 2004a, 2005, 2008). Although some members of this family of gram-negative, facultative anaerobic bacteria are spoilage organisms, many are also human pathogens. Escherichia, Proteus, and other members of this bacterial family are recognized as causing various types of egg rots (Florian and Trussell, 1956; Board et al., 1963; Board, 1966; Ricke et al., 2001). Enterobacteriaceae identified from the MO and OL facility in this study are listed in Table 1.

Enterobacteriaceae recovered on shelves in this study have been reported by other researchers who sampled eggs and equipment surfaces in shell egg processing plants (Haines, 1938; Florian and Trussell, 1956; Board, 1966; Moats, 1980; Davies and Breslin, 2003). Musgrove et al. (2008) reported that Escherichia, Enterobacter, Citrobacter, and Klebsiella were the most commonly encountered genera in shell egg processing plants. In the current study, Enterobacter and Escherichia accounted for 50% from the MO (29/49) and the OL facility (44/75). Moats (1979) reported that gram-negative rods such as Escherichia were commonly encountered on washed and unwashed eggs but no Enterobacteriaceae were recovered on processing equipment surfaces. In Moats’ study, samples were analyzed for the presence of aerobic microorganisms, not a particular bacterial family, genus, or species.

In sanitation surveys conducted more recently, Enterobacteriaceae were recovered from many surfaces within shell egg processing facilities; those that did and those that did not have direct contact eggs were found to be contaminated (Jones et al., 2003; Musgrove et al., 2004b). Prior to sanitation procedures being performed, nest run cart shelves, the floor under the farm belt (conveyor belt that transports eggs from the hen houses to the processing plant), the floor under the nest run loader (transfer eggs from flats to the processing line), and the drain near the washers were all contaminated with >2.0 log_{10} cfu/mL of swab diluent. After sanitation, only the nest run cart shelves and drain were contaminated at this level (Musgrove et al., 2004b).

Cleaning is the primary means of preventing the formation of microbial growth niches in processing facili-

ties. Because of its porous nature, wood can be very difficult to clean (Forsythe and Hayes, 2000; Agle, 2007). This is the primary reason that some sources recommend against using equipment with wooden surfaces in processing environments (Forsythe and Hayes, 2000). Dawson and others recently demonstrated cross-contamination with bread and bologna after contact with Salmonella-inoculated wood (Dawson et al., 2006). It has also been reported that Salmonella Typhimurium survives longer and is more difficult to kill when inoculated onto wooden cutting boards when compared with boards composed of less porous materials. (Abrisham et al., 1994; Gough and Dodd, 1998). Salmonella was identified from the MO and from the OL facility.

When microbial populations in these niches are allowed to thrive, biofilm may form. A biofilm is a community of sessile microorganisms characterized by cells that are irreversibly attached to a surface and that are not displaced by rinsing (Agle, 2007). Smooth or rough surfaces can be colonized and surfaces that retain moisture, such as untreated wood, increase chances of bacterial survival. Salmonella has been demonstrated to persist on wooden surfaces, including chopping blocks (Gough and Dodd, 1998; Davies and Breslin, 2003). Generally, eggs that are transported on nest run egg carts have not been washed. However, there are times, particularly on hectic days, when packaged eggs may be placed on the wooden shelves. Given the amount of moisture, degree of dirtiness, and levels of bacterial contamination (3.2 to 3.8 log_{10} cfu/mL of rinse), the opportunity for cross-contamination exists. On any processing day, eggs will be broken. At the OL facility, Enterobacteriaceae levels on bottom shelves were significantly higher than on top shelves perhaps because egg contents may accumulate as eggs on higher shelves are broken (2.0 vs. 4.0 log_{10} cfu/mL of swab). Even if eggs or packaging do not become contaminated while near the shelves, in some older facilities, not all equipment or personnel move from cleaner parts of the processing environment to dirtier areas. Hands of personnel may become contaminated while moving the carts and may spread microorganisms to other machinery and even to eggs themselves (Forsythe and Hayes, 2000).

This study has demonstrated that nest run cart shelves were consistently contaminated with Enterobacteriaceae, including Salmonella. Nest run carts are vital to efficient operation of many shell egg processing facilities. Although wooden shelves are inexpensive and durable, they do provide a sanitation challenge. If the shelves are painted, covered with other wood finishes, or constructed from different materials, they may be more easily sanitized. Stainless and zinc-plated carts specialized for use in commercial shell egg facilities are available (Forsythe and Hayes, 2000). Although more expensive, alternative nest run carts may be useful in reducing Enterobacteriaceae growth niches in the shell egg processing environment.
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REFERENCES