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# ***Pathogen Detection and Remediation for Safe Eating***

# The use of *Aeromonas* as a process indicator during swine carcass dressing and cutting

Running Title: *Aeromonas* spp. on swine carcasses

## ABSTRACT

Using starch ampicillin agar, qualitative and quantitative determinations of *Aeromonas* spp. were made at several sites during swine carcass dressing and cutting. *Aeromonas* spp. were observed at all sites surveyed. Levels increased during shackling and passage through the first and middle polisher/washers, and significantly decreased during the singeing steps. Passage through the final polisher/washer caused a small increase in levels in *Aeromonas* spp. and these levels then remained constant during the rest of the carcass dressing operation. *Aeromonas* spp. were also isolated from the room where the carcasses were cut into wholesale cuts and cuts for further processing. Presumptive *Aeromonas* spp. cultures isolated from the different sites were confirmed as belonging to the genus *Aeromonas* and then speciated using the biochemical scheme of Joseph and Carnahan; 81% of the cultures were identified as *A. hydrophila*. Since most isolates were *A. hydrophila*, determination of the origin of isolates from different sites in the processing plant must await utilizing molecular biotyping techniques on the cultures. These results indicate the *Aeromonas* spp. occurs extensively in the swine dressing environment and thus represents a possible public health hazard and potential spoilage concern. Changes in cleaning and sanitizing of equipment may be necessary during swine carcass dressing and cutting to guard against this pathogen.

**Keywords:** *Aeromonas* spp., process indicator, swine carcass dressing

## 1. INTRODUCTION

Members of the genus *Aeromonas* occur widely in various environments, particularly in aquatic environments. *Aeromonas* spp. are widely recognized as pathogens of fish and frogs and as a putative pathogen of humans in which they are often associated with diarrhea, especially in children. These bacteria can be isolated from a variety of foods including foods of animal origin<sup>5</sup>. *Aeromonas* spp. can also be isolated from the feces of various red meat animals and from poultry, though generally at a low incidence<sup>5</sup>. In addition, these bacteria can be isolated from various water sources including chlorinated and non-chlorinated drinking waters, marine, and river waters<sup>5</sup>.

Since *Aeromonas* spp. are sensitive to heat, acid, and chlorine<sup>5</sup>, their presence in different processed foods undoubtedly represents post processing contamination. In addition to their other characteristics, *Aeromonas* spp. are recognized psychrotrophic pathogens, i.e., capable of growth at 5°C, the temperature thought previously to prevent the growth of foodborne pathogens and the temperature which should extend the general bacteriological shelf life of refrigerated fresh and processed foods.

Borch et al.<sup>1</sup> have suggested that the presence of *Aeromonas* spp. could be useful as an indicator of processing equipment hygiene in swine slaughter plants. The authors also suggested that these bacteria can be endemic in swine slaughter facilities. Our objectives were to determine the incidence and level of *Aeromonas* spp. on swine carcasses at different sites during carcass dressing and cutting and on equipment used to process and transport swine carcasses and primal

cuts; to determine the effects of various processing steps on their levels; and to determine, if possible, the origins of any *Aeromonas* spp. detected and isolated.

## 2. MATERIALS AND METHODS

### 2.1. Collection of samples

Samples were obtained from a swine processing plant which slaughters between 800 and 900 swine/hr. The sequence of steps employed in the slaughter plant where the sampling was done is shown in Fig. 1. The surfaces of the carcass during dehairing and shackling (gambrel table) were sampled by swabbing areas of approximately 100 cm<sup>2</sup> without delimiting an area by a template<sup>2</sup>. Five samples were obtained on each of 4 days. For swine carcasses, the bellies were sampled, without the delimitation of a surface area by rubbing a sterile Whirl-Pak swab (Nasco, Fort Atkinson, WI) moistened with 10 ml of 0.1% w/v peptone water over approximately 100 cm<sup>2</sup> of the surface of each belly. On each of 4 days, 10 bellies were sampled before the first polisher, after the final singer, after the final polisher, and after the final rinse. The next day, 10 carcasses were similarly sampled after overnight chilling (18-24 hrs at 2°C).

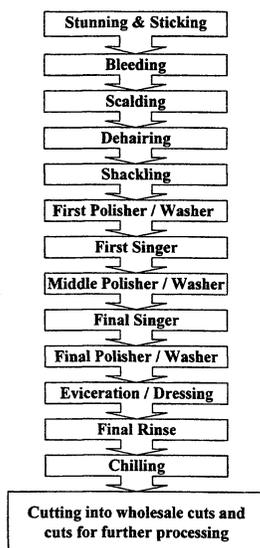


FIG. 1 Steps in Swine Slaughter and Carcass Dressing

After the swabs obtained were stomached for 2 min, 0.5 ml portion of the fluid from the stomached samples or, when increased sensitivity was required, serial dilutions, were surface plated onto and starch-ampicillin agar (SAA; 4) plates. Presumptive colonies, typically 3-5 mm in diameter and yellow to honey colored, were picked. After incubation at 28°C for 24 h, the SAA plates were flooded (ca. 5 ml) with Lugol iodine solution and amylase-positive colonies (those with peripheral clear zones of starch hydrolysis) were scored as presumptive *Aeromonas* spp. If the colony selected was amylase positive, the culture was further processed for confirmation and identification as described below.

### 2.2. Incidence of *Aeromonas* spp. during carcass dressing

A total of 45 surface samples were taken using Rodac plates with SAA medium. Five samples were taken from each of the following areas: dehairer, gambrel table, carcass before first polisher, carcass after the final singer, wall inside the final polisher, carcass after the final polisher and carcass before the chiller. All plates were subsequently incubated at 28 °C for 24 h before isolation and identification.

### 2.3. Incidence of *Aeromonas* spp. during carcass cutting process

The surface of the carcass breaking and cutting belts were sampled by rubbing a sterile swab moistened with 0.1% (w/v) peptone water over approximately 100 cm without the delimitation of an area by a template. Five swabs were taken from each of six process belts: main, ham, shoulder (butt or picnic), loin, belly and rib packing lines. A total of 30 swabs were stomached for 2 min and the fluid from each stomached sample was filtered through a membrane filter (47 mm diameter, 0.45 µm pore size) (Nalgene) before being placed onto the SAA plates for 24 h incubation at 28°C.

#### 2.4. Confirmation of isolates

Presumptive *Aeromonas* spp. isolated from equipment and carcass were confirmed by using the following protocols, tests and media: Gram stain, oxidase, catalase, confirmation media (esculin hydrolysis and gas formation from glucose), resistance to vibriostatic agent O/129, resistance to ampicillin and cephalothin, API 20E strips (bioMerieux Vitek, Inc., Hazelwood, MO). The characteristics of the isolates were compared against those described by Joseph and Carnahan<sup>3</sup> and Popoff<sup>6</sup> to speciate isolates. The incubation temperature for the biochemical tests was 37°C.

### 3. RESULTS AND DISCUSSION

Work currently in progress at this plant indicates that singeing of the carcasses substantially reduces total bacterial numbers on carcasses (data not shown); however, there are small increases in total numbers of bacteria introduced onto the carcasses by different operations after the singeing operation (data not shown). Numbers of *Aeromonas* spp. were quantitated at several steps during slaughter and dressing of swine carcasses. These data are shown in Tables 1 and 2. The data in Table 1 indicate that there were *Aeromonas* spp. present on swine carcasses very early in the dressing operation. The counts of *Aeromonas* spp. ranged from 0.49 CFU/cm<sup>2</sup> before dehairing and increased to 1.8 CFU/cm<sup>2</sup> after dehairing; similarly, the counts increased after shackling. This was not unanticipated since, as will be shown below, *Aeromonas* spp. were detected on the gambrel table, the site where the animals are shackled.

Table 1. Numbers of *Aeromonas* spp. detected on carcasses during the early steps in swine carcass dressing.

Step Sampled During Swine Carcass Dressing			
Before Dehairing	After Dehairing	Before Shackling	After Shackling
0.49*	1.8*	0.3*	1.6*

\*Counts given as CFU/cm<sup>2</sup> on starch ampicillin agar; belly swabs, average of 20 carcasses.

The data in Table 2 indicate that *Aeromonas* spp. were present on swine carcasses essentially throughout the entire carcass processing sequence. These data support the observation that singeing reduces the level of *Aeromonas* spp. along with the total bacterial levels. However, this dramatic decrease is short lived in that the level of *Aeromonas* spp. on the carcasses increases during their passage through the final polisher/washer. Because of mechanical restrains, quantitation was not possible, but the presence of *Aeromonas* spp. was detected on the inside surfaces of that final polisher/washer. After passage through the final polisher/washer, levels of *Aeromonas* spp. on the carcass then remained relatively low during the rest of the steps involved in carcass dressing. While quantitative data were not obtained, *Aeromonas* spp. were detected and isolated from the equipment used in the final cutting of the carcass into wholesale cuts and cuts for further processing.

Table 2. Numbers of *Aeromonas* spp. detected at different sites during the dressing and handling of swine carcasses.

Site Sampled During Swine Carcass Dressing and Handling				
Before First Polisher	After Final Singeer	After Final Polisher	After Final Rinse	After Chilling
8.7*	0.3*	2.8*	1.1*	1.6*

\*Counts given as CFU/cm<sup>2</sup> on starch ampicillin agar; belly swabs, average of 40 carcasses.

During both the quantitative portion of this study and the incidence survey, cultures of presumptive *Aeromonas* spp. cultures were isolated, purified using standard techniques and verified as belonging to the genus *Aeromonas* and then identified biochemically using the scheme of Joseph and Carnahan<sup>3</sup> as well as the characteristics traits of the genus<sup>6</sup>. All isolates were: Gram negative rods, oxidase and catalase positive, resistant to the vibriostatic agent O/129, and amylase positive. The biochemical scheme given by Joseph and Carnahan<sup>3</sup> allow speciation of all the currently known hybridization group of the genus *Aeromonas*. Using their scheme, 82% of our isolates were identified as *A. hydrophila* (Table 3). Small numbers of *A. veronii* *bv sobria* and *A. veronii* *bv veronii* were also identified; almost 10% of the isolates could not be speciated using current biochemical identification schemes. Since the prevalent bacterium isolated was *A. hydrophila*, no determination could be made as to the source of these strains introduced onto the carcasses.

Table 3. Speciation of *Aeromonas* strains isolated from various sites during swine carcass dressing and cutting.

Identity of isolate	No. of isolates (%)	Site from which culture isolated (Number of isolates)
<i>A. hydrophila</i>	45 (81.8%)	Dehairer (3), Gambrel table (1), Carcasses before the first polisher (3), Wall inside last polisher (8), Carcasses after last polisher (1), Carcasses before chiller (4), Main line*(7), Ham line (9), Shoulder line (5), Loin line (2), Rib packing line (2)
<i>A. veronii</i> bv <i>sobria</i>	4 (7.3%)	Dehairer (1), Gambrel table (1), Wall inside last polisher (1), carcasses after last polisher (1)
<i>A. veronii</i> bv <i>veronii</i>	1 (1.8%)	Carcasses before the first polisher (1)
Unspeciated	5 (9.1%)	Dehairer (1), Gambrel table (1), Loin line (1), Belly line (2)

\*Any site preceded by term line is that part of the operation where the carcass is cut into various wholesale cuts and cuts for processing into different cured products.

Overall, our findings indicate that *Aeromonas* spp. are present throughout the carcass dressing operation. Therefore, changes in cleaning and sanitizing may be necessary to eliminate these bacteria from the carcass dressing and handling operation. The results of this study stress the need for utilizing one of the molecular biotyping techniques such as ribotyping on these strains to determine the source and origin of these bacteria. This information will have implications in designing HACCP plans that would ensure safety against *Aeromonas* spp. during swine slaughter and carcass dressing operations.

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