Antimicrobial Effects of *Pseudomonas aeruginosa* on Survivability and Recovery of *Campylobacter jejuni* on Poultry Products

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ABSTRACT Three types of poultry products representing differences in skin coverage were tested to determine the ability of *Pseudomonas aeruginosa* to inhibit growth of *Campylobacter jejuni*. Processed ready-to-cook poultry carcasses were obtained from the Poultry Research Unit at Auburn University and were not subjected to any treatment to reduce or eliminate the native microflora on the carcasses. Carcasses were cut into wing sections (drumette, flat, tip), split breast pieces (with and without bone), and boneless, skinless breast pieces. Equal numbers of the 3 product types were subjected to 1 of 6 treatments: 1) uninoculated, 2) *C. jejuni* only, 3) *P. aeruginosa* type 1 only, 4) *P. aeruginosa* type 2 only, 5) *C. jejuni* + *P. aeruginosa* type 1, or 6) *C. jejuni* + *P. aeruginosa* type 2. Products were inoculated at 10^4 to 10^5 cfu. Postinoculation, equal numbers of product type were also subjected to the following: 1) aerobic or vacuum packaging, 2) storage temperature of 4 or 10°C, and 3) storage of 0, 1, 2, 3, or 4 d. Products were sampled after storage duration to determine the population of *C. jejuni* and *P. aeruginosa*. Individual pieces were rinsed with 50 mL of buffered peptone water. The recovered rinse was used to make appropriate dilutions and spiral plated onto Campy-Cefex and *Pseudomonas* P agars. Campy-Cefex plates were incubated microaerophilically at 42°C for 48 h, whereas *Pseudomonas* P plates were incubated aerobically at 37°C for 24 to 48 h. Random suspect colonies on Campy-Cefex plates were confirmed by cell morphology when viewed under microscopic examination. Suspect colonies on *Pseudomonas* P plates produced a blue color in the medium indicative of glycerol reduction. At both 4 and 10°C, neither type of *P. aeruginosa* inhibited the growth or survival of *C. jejuni* compared to plates that were inoculated with *C. jejuni* only.

Key words: *Campylobacter*, *Pseudomonas*, poultry, antimicrobial

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INTRODUCTION

In the United States and many other developed countries, the leading pathogen causing acute gastroenteritis is *Campylobacter jejuni* (Butzler and Skirrow, 1979; Blaser et al., 1983; CDC, 2001). Previous studies show that this organism is easily transmitted from the environment to the consumer by poultry products (Hopkins and Scott, 1983; Harris et al., 1986b) and that a distinct epidemiological link exists between the consumption of improperly prepared poultry meat and human illness (Bryan and Doyle, 1995). Even though *Campylobacter* has reservoirs in the environment (Conner et al., 2001; Hargis et al., 2001), the major reservoir for *Campylobacter* is the intestinal tract of poultry (Oosterom et al., 1983a), especially the ceca and crop (Oosterom et al., 1983b; Hargis et al., 1995; Franco and Williams, 2001). Because both of these harbor sites can be ruptured during the initial processing of the chicken carcass (Hargis et al., 1995), the organism may be transferred to the skin and meat of the carcass. Although Davis and Conner (2000) found a relatively low incidence of *Campylobacter* on skinless retail poultry products, they also found that once *Campylobacter* has been introduced onto the skinless product, it will survive very well in the absence of competing microflora (Davis and Conner, 2002). Because the poultry processing environment is not sterile and many other types of bacteria are located on poultry skin and meat, Mai (2003) studied the effects of various poultry microbial isolates on the survivability of *C. jejuni*. Results from this study show that many psychrotrophic spoilage organisms commonly associated with the poultry carcass reduce the numbers of *C. jejuni* in both broth and agar cultures by as much as 5.8 $\log_{10}$ cfu/mL (Mai, 2003). The objective of this study was to determine effects of *Pseudomonas* isolates previously determined to inhibit the growth of *C. jejuni* when coinoculated on various types of poultry products.

MATERIALS AND METHODS

Poultry Products

For this experiment, poultry wings, skin-on split breast pieces (with and without bone), and skinless breast pieces

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Table 1. Mean and significant difference comparison of *Campylobacter jejuni* populations recovered from products subjected to varying storage temperatures and atmospheric conditions

Item	Mean *C. jejuni* population (log_{10} cfu/mL)	Minimum significant difference ($\alpha = 0.05$)
Sample day (D)	NS[1]	0.1544
0	3.13	
1	3.18	
2	3.10	
3	3.15	
4	3.23	
Storage temperature (S)	***	0.064
4°C	3.37[a]	
10°C	2.94[b]	
Treatment (T)	NS	0.1091
C. jejuni only	3.14	
C. jejuni + *Pseudomonas aeruginosa* type 1	3.19	
C. jejuni + *P. aeruginosa* type 2	3.15	
Atmospheric condition (A)	NS	0.0748
Aerobic	3.18	
Vacuum-packaged	3.14	
Product type (P)	NS	0.109
Wing	3.19	
Skin-on split breast	3.17	
Skinless breast	3.15	
Interactions		Pooled SEM
S × T	*	0.0330
T × A	*	0.0330
D × S × T	***	0.0738
D × S × A	*	0.0603
D × T × P	***	0.0904
S × T × P	***	0.1278
T × A × P	*	0.0572
D × S × T × A	**	0.1044

[a,b]Differing superscripts within a main effect indicate significant differences ($P \leq 0.05$).

[1]Not significant at $P > 0.05$.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

were used to provide poultry products representing differing areas of skin coverage. All product types were processed at the Auburn University Poultry Research Unit. The carcasses were processed approximately 1 mo before the start of the experiment and kept at 0°C until 1 d before the beginning of the experiment. The wings (drumette, flat, tip) were used for complete skin coverage. Skin-on split breast pieces were used for partial and varying skin coverage, and skinless breast pieces were used for zero skin coverage. Different skin coverages were used to determine if survivability and recovery of *C. jejuni* and *Pseudomonas aeruginosa* were affected by skin coverage.

Storage Conditions

Two atmospheric conditions (aerobic and vacuum) were used for product storage. Products stored in aerobic conditions were placed in Styrofoam trays (Dow Chemical Co., Midland, MI) and covered with Cryovac film (Sealed Air Corp., Elmwood Park, NJ). Vacuum-packaged products were packaged using the retail Foodsaver vacuum-packaging machine model 820 and Foodsaver quart volume bags (Jarden Store, San Francisco, CA). Storage temperatures were 4 and 10°C for up to 4 d postinoculation.

Bacterial Treatments

Sample inoculation consisted of the following treatments: 1) uninoculated product types for control and 2) *C. jejuni* only, 3) *P. aeruginosa* type 1 only, 4) *P. aeruginosa* type 2 only, 5) *C. jejuni* + *P. aeruginosa* type 1, or 6) *C. jejuni* + *P. aeruginosa* type 2. Three samples of each product type were inoculated with 1 of the treatments for d 0 (immediate) testing. Day-zero testing consisted of only aerobic samples. Testing for d 1 to 4 used 3 samples of each product type inoculated with 1 of the 6 treatments and placed in 1 of the 2 atmospheric conditions (12 each of each sample type tested per day). Initial inocula were made by using plate-grown cultures to make a McFarland 0.5 turbidity dilution. Initial populations for 4°C storage were as follows: *C. jejuni*, 6.00×10^5 cfu/mL; *P. aeruginosa* type 1, 4.85×10^5 cfu/mL; and *P. aeruginosa* type 2, 5.00×10^5 cfu/mL. Initial populations for 10°C storage were as follows: *C. jejuni*, 8.50×10^4 cfu/mL; *P. aeruginosa* type 1, 1.72×10^4 cfu/mL, and *P. aeruginosa* type 2, 3.56×10^4 cfu/mL. All bacterial inoculations were given at 1 mL spread over each piece of poultry product via pipette. These inoculations were allowed to set for 5 min before packaging. Although concentrations of *Campylobacter* and *Pseudomonas* are not typically this high on freshly pro-

Table 2. Mean and significant difference comparison of *Pseudomonas aeruginosa* populations recovered from products subjected to varying storage temperatures and atmospheric conditions

Item	Mean *P. aeruginosa* population ($\log_{10}$ cfu/mL)	Minimum significant difference ($\alpha = 0.05$)
Sample day	***	0.4070
0	4.19[a]	
1	7.07[b]	
2	6.88[c]	
3	7.64[d]	
4	8.34[e]	
Storage temperature	***	0.1959
4°C	6.09[a]	
10°C	7.61[b]	
Treatment	NS[1]	0.5512
Uninoculated	6.72	
Campylobacter jejuni	6.73	
P. aeruginosa type 1	6.83	
P. aeruginosa type 2	6.89	
C. jejuni + *P. aeruginosa* type 1	6.93	
C. jejuni + *P. aeruginosa* type 2	6.98	
Atmospheric condition	***	0.2158
Aerobic	6.54[a]	
Vacuum-packaged	7.23[b]	
Product type	NS	0.3204
Wing	6.80	
Skin-on split breast	6.92	
Skinless breast	6.81	

[a–e]Differing superscripts within a main effect indicate significant differences ($P \leq 0.05$).

[1]Not significant at $P > 0.05$.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

cessed poultry carcasses, these inoculation concentrations were chosen to give a 1-to-1 concentration of the target bacteria and allow for enumeration if the results had matched that of Mai (2003).

Enumeration

When the sampling date arrived, replicate product types were removed from the packaging and placed in separate, sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI). Each bag received 50 mL of buffered peptone water and was shaken vigorously for 1 min. Ten milliliters of this solution was used to make an "original" plate, whereas 1 mL was used to make serial dilutions up to 10^{-5}. Appropriate dilutions were then spiral plated onto Campy-Cefex and *Pseudomonas* P agars. Campy-Cefex and *Pseudomonas* P plates were both made in-house from media components obtained from Difco Laboratories (Detroit, MI) and Neogen (Baltimore, MD). Campy-Cefex plates were incubated microaerophilically for 48 h at 42°C. *Pseudomonas* P plates were incubated aerobically for 24 h at 37°C. Suspect colonies from Campy-Cefex agar plates were confirmed by cell morphology under gross microscopic examination. Suspect *P. aeruginosa* colonies produced a color change from clear to blue on *Pseudomonas* P agar. Plates were counted using a laser counter.

Data Analysis

The experimental design was as follows. Total numbers for each product type were 324 (3 samples × 6 inocula × 2 atmospheres × 2 temperatures × 4 sampling days + 36 each for d 0 aerobic testing only), observed for *Campylobacter* and *Pseudomonas* colony growth. Counts were then converted to base-10 logarithm values and subjected to PROC GLM and Tukey statements of the SAS system (SAS Institute, 1997). Random samples from *Pseudomonas* P plates were then subjected to ribosomal RNA analysis (ribotyping) to determine if the *Pseudomonas* colonies were the same as the inoculated Pseudomonas, because the sample types had not been exposed to any procedure that would eliminate native microflora.

Isolate Characterization

Ribosomal RNA analysis was performed using a DuPont Qualicon riboprinter (DuPont Inc., Wilmington, DE). Isolates were sampled using quality-assured materials from DuPont and were compared using the EcoR1 DNA analysis. Isolate identification was assumed to be correct if the probability was 75% or above.

RESULTS AND DISCUSSION

A summary of the statistical analysis of main effects for *C. jejuni* populations in this experiment is given in Table 1. Because there were no *C. jejuni* populations recovered from treatments not inoculated with *C. jejuni*, statistical analysis was performed only for those treatments in which *C. jejuni* was inoculated and, thus, recovered. For surviving *C. jejuni* populations, sample day (day postinoculation), atmospheric condition, product type, and

Table 3. Significant main effect interactions associated with *Pseudomonas aeruginosa* survival

Interaction	Significance level	Pooled SEM
Day × temperature	***	0.0385
Day × treatment	***	0.0668
Temperature × treatment	***	0.0422
Day × temperature × treatment	***	0.0944
Day × atmosphere	***	0.0385
Temperature × atmosphere	***	0.0243
Day × temperature × atmosphere	***	0.0545
Day × temperature × treatment × atmosphere	*	0.1335
Day × product type	***	0.0472
Temperature × product type	**	0.0299
Day × temperature × product type	*	0.0668
Treatment × product type	***	0.0517
Day × treatment × product type	***	0.1156
Temperature × treatment × product type	***	0.0731
Day × temperature × treatment × product type	***	0.1635
Temperature × atmosphere × product type	*	0.0422
Day × temperature × atmosphere × product type	*	0.0944
Treatment × atmosphere × product type	*	0.0731
Day × treatment × atmosphere × product type	***	0.1635
Temperature × treatment × atmosphere × product type	**	0.1034
Day × temperature × treatment × atmosphere × product type	*	0.2313

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

treatment had no significant effect ($P > 0.05$). However, storage temperature did have significant effects ($P \leq 0.001$). *Campylobacter jejuni* did not survive as well in the warmer 10°C environment as it did in the cooler 4°C environment. This suggests that *C. jejuni* may have some adaptive characteristics that allow it to survive at cooler temperatures, and this is also consistent with studies conducted in Norway, in which thermotolerant species of *C. jejuni* survived well at 4°C (Franco and Williams, 2001). It is also known that *Campylobacter* can be cultured from frozen poultry meat (Nachamkin and Blaser, 2000).

Main effects for *Pseudomonas* are shown in Table 2. For this organism, treatment and product type were not significant effects ($P > 0.05$). Sample day, storage temperature, and atmospheric condition were all significant ($P \leq 0.001$). Although surviving populations of *P. aeruginosa* were not a main focus of this study, the nonsignificance of treatment should be noted. No significant difference was observed between treatments that were not inoculated with *P. aeruginosa* and those that were. Apparently, surviving populations of other Pseudomonads and other spoilage organisms were already present on the products sampled. This observation, when considered with the findings that many bacteria ribotyped from the *Pseudomonas* P agar plates were not the *P. aeruginosa* inoculated [*Serratia liquefaciens, Acinetobacter baumannii, P. aeruginosa* (3 types), *Stenotrophomonas maltophila, Pseudomonas fluorescens* (2 types), *Staphylococcus epidermidis, Hafnia alvei*], suggests that a complex microbial ecology exists on poultry skin and meat and that the 2 isolates of *P. aeruginosa* used for this study may not have competed well in this environment.

Factorial tables of the interactions of the main effects for *C. jejuni* and *P. aeruginosa* can be found in Tables 1 and 3, respectively. There were many significant interactions, including a 4-way interaction of main effects for *C. jejuni* populations and a 5-way interaction among all main effects for *P. aeruginosa* populations. Although there are sporadic differences, *C. jejuni* survived at much the same rate whether or not it was subjected to either of the *P. aeruginosa* cultures. This finding contrasts with those of Mai (2003). Her findings showed that in broth and agar media, the 2 cultures of *P. aeruginosa* used in this study decreased *C. jejuni* populations by 5.765 and 4.575 logs (Mai, 2003). This suggests that although *P. aeruginosa* may affect the survival of *C. jejuni* in isolated populations, the microbial dynamic of poultry meat may not lend itself to this phenomenon. These interactions, along with the survival of *C. jejuni* throughout the experiment and the findings of other spoilage microorganisms, further suggest that the microbial dynamic on poultry skin and meat is very complex, and although some spoilage microbes may be antagonistic to *C. jejuni* in isolation, this antagonism may be muted on poultry products.

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