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The Incidence of *Escherichia coli* on Beef Carcasses and Its Association with Aerobic Mesophilic Plate Count Categories During the Slaughter Process†

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ABSTRACT

An analysis of 535 prefabricated beef carcass samples taken in three processing plants demonstrated an association between the mesophilic aerobic plate count (APC) class and the incidence of obtaining an *Escherichia coli*–positive sample. Beef carcasses were sampled from three separate plants; one was a fed-beef processing plant and the other two were cow/bull plants. Samples were obtained by sponging and were analyzed for APC and *E. coli*. When samples were classified into four APC levels or classes (class 1: <2, class 2: ≥2 and <3, class 3: ≥3 and <4, and class 4: ≥4 log CFU/cm^2), a trend indicating that samples from higher APC classes were more likely to be positive for *E. coli* biotype 1 was observed. Of the APC class 4 samples (≥4 log CFU/cm^2), 88% were positive for the presence of *E. coli*, as opposed to 21% in APC class 1 (<2 log CFU/cm^2). Univariate chi-square analysis of the resulting contingency tables from reclassified data (class 1: <2, class 2: ≥2 and <3, and class 3: ≥3 log CFU/cm^2) indicated a strong association between APC class and the incidence (presence or absence) of an *E. coli*–positive sample. Using multivariate analysis to account for influences of plant and within plant processing site, the data indicated a strong positive linear trend between the presence of *E. coli* and the APC class.

As part of its pathogen reduction program, the United States Department of Agriculture, Food Safety and Inspection Service will require animal processors to develop and implement approved hazard analysis and critical control point plans *(9)*. As a part of the plan, plants must document verification of process control by testing finished carcasses for the presence of biotype 1 *Escherichia coli* on carcasses.

Under normal beef processing conditions, removing the hide of a healthy animal presents a carcass surface that is essentially sterile. In a short time, as processing proceeds, the carcass will become contaminated, to varying degrees, with microorganisms. The source of major microbial contamination is feces from the hide, hair, and hooves of the animals. Gross microbial contamination of carcasses is largely attributable to contamination with feces from the hide, hair, hooves, or ruptured gut *(2, 8)*. Carcass contamination from feces can be visible or invisible, but in either case, residual bacteria are present. The intestinal tract is considered the major reservoir, and animal feces the main route of transmission, of several human bacterial enteric pathogens including *E. coli* O157:H7 and other verotoxigenic *E. coli*. In the United States, visible carcass fecal contamination is only removable by knife trimming or, more recently, by the use of a steam vacuum device for a specified area *(6, 23)*. To date, detecting invisible sources of microbial contamination remains problematic.

The fourth principle of hazard analysis and critical control point programs *(21)* is monitoring of the critical control points. For food processing, this should entail measurements that are collected in real time (e.g., temperature, pH). To date there are no real-time, on-line microbial tests that function at a level of sensitivity or low limit of detection needed to detect viable microbial contaminants. Microbial verification of processing has relied on tests that still only provide data that are retrospective in nature. To utilize a microbial test as a critical control point monitor of the preboning stages of beef carcass processing, the resulting data must be useable for corrective action to be executed before the carcass is chilled.

The use of indicator bacteria as a means to detect the presence of fecal contamination has a long history. Several fecal indicators, both biological and chemical, have been reported *(16, 22)*. These include fecal coliforms, fecal enterococci (group D streptococci), and fecally derived cholesterol breakdown products, such as coprostanol and skatole.

The utility of the standard mesophilic aerobic plate count (APC) as a microbial monitor of processing and as a predictor of ultimate food quality has been studied thoroughly *(2, 15, 16)*. The APC is widely used as a means to

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assess the shelf life and stability or the overall microbial quality of raw ingredients *(15)* as well as finished products. However, the use of the APC as an indicator of safety, or of the presence of specific pathogens, is generally not accepted *(15, 17, 18)*.

In light of the hypothesis purported in this study, the usefulness of the APC should be reexamined as a means of process monitoring. The origin of major microbial contamination on carcasses is feces deposited onto an initially sterile surface via the hide, hooves, hair, or dust. It can be deduced that high microbial loads on a carcass, prior to fabrication, would indicate a high likelihood of fecal contamination.

The purpose of this research was to ascertain the association between the APC, or load of a carcass surface, and the likelihood of a carcass surface sponge sample testing culture positive for the presence of *E. coli*. This information could be useful in developing real-time monitors of fecal contamination.

MATERIALS AND METHODS

Sampling plan. The numbers of sponge samples collected from each plant are presented in Table 1. Randomly selected carcasses from one large fed-beef (young grain-fed steers and heifers) plant (designated plant "A") and from two medium-sized cow/bull processing plants (designated plants "B" and "C") were selected at the stages of postevisceration/prewasher (designated prewash); immediately post–water wash (designated postwash); and from the 4°C chiller (designated chilled). All carcass washes were performed with potable water with no added antimicrobial.

Carcasses from plant A were randomly selected from the prewash and postwash (prechilled) stages. Carcass surface areas ranging in size from 81 to 150 cm^2 were sponge sampled (see Sampling method) *(19)*.

In plants B and C, three 100-cm^2 sites (flank, brisket, and rump) on one side of the selected carcass were sponged sampled as described (see Sampling method). For the three-site sampling (plants B and C), the flank, brisket, and rump were sampled using a single sponge per carcass.

From plant B, 30 carcasses were sampled on each day for 3 days on two consecutive weeks (except for the last day of the second week on which 25 samples were taken), resulting in a total of 175 carcasses sampled. From plant C, 20 carcasses were sampled at each location (prewash, postwash, and chilled) for a period of 3 days at the rate of 15 per day per location over 4 days spread out over two consecutive weeks.

Sampling method. Samples were taken using a sterile, microbiological sampling sponge (Speci-Sponge, NASCO, Fort Atkinson, Wis.) packaged in a Whirlpak bag. The sponge was moistened with 25 ml of a sterile sponge solution composed of 0.085% (wt/vol) NaCl and 0.05% (vol/vol) Tween 20 adjusted to pH 7.8. The solution was expressed from the sponge as it was removed from the Whirlpak bag using a sterile glove. The sponge was wiped firmly over the sample area 10 times in both the vertical and horizontal direction. The sponge was then placed into the bag containing the residual sponge solution and held at 5°C. Analyses were performed within about 2 to 3 h of taking the sample. The efficacy of this method has been reported previously *(7, 20)*.

Microbiological analysis. Samples were diluted in buffered peptone water (Difco Laboratories, Detroit, Mich.), and 1-ml portions were plated on Aerobic Count Petrifilm (3M, St. Paul, Minn.) and *E. coli* Petrifilm (3M). Inoculated Petrifilms were incubated and counted as per manufacturer's instructions.

Statistical analysis and univariate data analysis. Standard mesophilic APCs were converted to the log of the counts per square centimeter of surface tissue sampled. The APCs were classified as follows: class 1 (log CFU/cm^2 <2), class 2 (log CFU/cm^2 ≥2 amd <3), class 3 (log CFU/cm^2 ≥3 and <4), and class 4 (log CFU/cm^2 ≥4). Contingency tables of data were constructed by combining APC class 4 into class 3 since four class divisions were not always sufficient to fill all contingency table cells adequately due to the low numbers of class 4 samples. The strength of association was estimated by using a chi-square analysis performed with the PROC FREQ procedure from SAS (SAS Institute, Cary, N.C.).

Statistical modeling and logistic regression. The strength of association between *E. coli* isolation from a beef carcass and each potential independent variable was also measured using logistic regression. Logistic regression is applicable when the outcome is dichotomous (positive or negative for biotype 1 *E. coli*) and the independent variables are either categorical or interval (APC classifications), as in this study. Univariable and multivariable logistic regression generated odds ratios (ORs) with 95% confidence intervals *(10)* and Wald's test *P* values for statistical significance of the ORs. An OR approximates how much more likely it is for the dichotomous outcome to occur (i.e., *E. coli* isolation) among those with exposure to a given factor compared to those without exposure. The OR can vary from zero to positive infinity; if the OR equals one, then there is no difference in outcome among those with and without factor exposure. An OR greater than one indicates increased outcome likelihood; ORs less than one indicate decreased outcome likelihood. For categorical variables, the OR represents the odds of outcome for each exposure category

TABLE 1. *Sources of samples used in this study and the distribution of APC classifications*[a]

Plant	Plant type	Process location	*n*	% APC class 1	% APC class 2	% APC class 3	% APC class 4
A	Fed Beef	Prewash	90	11	52	37	0
C	Cow/bull	Prewash	60	2	57	33	8
A	Fed Beef	Postwash	90	21	67	12	0
C	Cow/bull	Postwash	60	3	67	28	2
B	Cow/bull	Chilled	175	74	23	2	1
C	Cow/bull	Chilled	60	28	57	15	0
A, C	(see above)	All prewash	150	7	54	35	4
A, C	(see above)	All postwash	150	14	67	19	<1
B, C	(see above)	All chilled	235	62	31	6	<1
A, B, C	(see above)	All locations	535	33	48	18	1

[a] See Materials and Methods (univariate analysis) for class definitions.

relative to the chosen reference condition. For continuous level variables, the OR is the multiplicative change in odds of outcome per unit increase in the independent variable.

Data were analyzed first by univariable and then by multivariable methods. For all analyses, the outcome of interest was isolation of *E. coli* (presence or absence) from the beef carcass. For univariable analysis, contingency tables of outcome versus each independent variable were created and evaluated by simple logistic regression using a commercial statistics software package (EGRET, Statistics and Epidemiology Research Corp., Seattle, Wash.). The chi-square test for linear trend was also used to assess dose response to ordinal exposure variables with more than two levels. A public domain software package was used to perform the tests for trend (EpiInfo, version 6.02, Centers for Disease Control and Prevention, Atlanta, Ga.).

To statistically adjust the main effects of each independent variable for differences in the distribution of and associations among the independent variables, multiple logistic regression was used (EGRET). Multivariable modeling has the advantage of looking at the data as a whole, which minimizes the loss of power that occurs when associations between specific data subsets are examined. Explanatory variables associated with the outcome at $P < 0.25$ by simple logistic regression with Wald's test or on the chi-square test for trend were used to construct the multivariable logistic models. First, a main effects model was constructed in which APC was specified as a three-level categorical variable (Model I). The multivariable modeling process was repeated with APC specified as a continuous variable (log APC per square centimeter). This dual modeling approach permitted examination of the adjusted association of the presence or absence of *E. coli,* with APC considered as either a categorical (Model I) or continuous variable (Model II). The goodness of fit for Models I and II was estimated by calculating the decile-based Hosmer and Lemeshow *C* statistic *(14).* This summary goodness-of-fit test estimates how the model describes the observed data.

RESULTS

Distribution of samples, simple chi-square and univariable analysis. The distribution of carcass sponge samples into APC classes 1, 2, 3, and 4 was 33, 48, 18, and 1%, respectively. A total of 237 of the 535 (44.3%) in the dataset were positive for biotype 1 *E. coli.* The distribution of biotype 1 *E. coli*–positive samples into APC classes 1, 2, 3, and 4 was 22, 49, 71, and 88%, respectively. A qualitative examination of the total combined dataset as well as the distribution (Table 1) and univariate chi-square analysis (Table 2) indicated that higher APC levels were associated with a greater percentage of samples testing positive for *E. coli* in samples taken after the spray wash step. Considering the data in its entirety, a strong relationship was indicated for the incidence of *E. coli*–positive samples as the APC class increased; the inverse was also apparent.

Changing the interval size of the APC classes is possible; however, it resulted in a loss of statistical power as well as several analytical cells of the contingency table that were below the needed sample number (data not shown). The same drawbacks occurred when breaking the data into individual processing plant datasets. This observation led to the use of logistic regression models.

Chi-square analysis of the associations between APC class and the incidence of obtaining an *E. coli*–positive sample from a postwashed sample (Table 2) indicated a strong linear trend ($P < 0.005$) between these two variables when examined by sample type (postwash, chilled, or combined datasets), with no indication of any nonlinear relationship ($P > 0.1$). Virtually all differences in the frequency or percent positive of an *E. coli* sample were taken into account by this linear trend relationship to APC class outside of the prewash stage. Slope calculations indicated an increase of 28% ± 2.3% per APC class score.

Plant source, carcass location, and APC (as continuous, three-level, or four-level categorical) showed significant simple association with *E. coli* isolation from beef carcasses (Table 3a). When carcass location and APC as a three-level or four-level categorical variable were considered as ordinal variables, the chi-square test for trend was also significant (Table 3a). Likelihood of *E. coli* isolation decreased in linear fashion from prewash to postwash to chilled carcass location. Conversely, likelihood of *E. coli* isolation increased linearly as APC class increased.

Multivariable analysis. Because plant source, carcass location, and APC were all important at the univariate level, all three variables were included as factors in multivariable logistic regression models. Plant source, carcass location, and APC remained significant whether APC was specified as a three-level categorical variable (Model I; Table 3b) or as a continuous variable (Model II; Table 3c). The adjusted likelihood of *E. coli* isolation from a beef carcass increased

TABLE 2. *Univariate contingency table and chi-square (χ^2) test for association and trend between APC class and isolation of* E. coli *biotype 1 from beef carcass sponge samples* (n = *535*)[a,b]

Process location	*n*	*E. coli* present	APC class 1	APC class 2	APC class 3	Total χ^2 (*P*)	χ^2 for linear trend (*P*)
Prewash	150	Yes	9	54	44	2.036 (0.360)	0.020 (0.658)
		No	2	27	14		
Postwash	150	Yes	5	47	19	8.513 (0.014)	8.44 (0.004)
		No	16	53	10		
Chilled	235	Yes	25	24	11	25.310 (<0.001)	22.61 (<0.001)
		No	121	50	4		
Combined	535	Yes	38	125	74	71.385 (<0.001)	71.22 (<0.001)
		No	140	130	28		

[a] See Materials and Methods (univariate analysis) for class definitions.

[b] APC classes were collapsed into three classes to accommodate cells with inadequate numbers for chi-square analyses.

TABLE 3a. *Univariate contingency table and simple logistic regression for association of various factors with* E. coli *isolation from 535 beef carcass sponge samples*

Independent variable	*E. coli* positive (%)	*E. coli* total	Odds ratio	Odds ratio with 95% confidence intervals	Wald's test *P* value	Chi-square for linear trend (*P* value)
Plant source						NA
A (fed cattle)	85 (47.2)	180	1.00	NA [a]	Reference	
B (cow/bull)	36 (20.6)	175	0.29	0.18 to 0.46	<0.001	
C (cow/bull)	116 (64.4)	180	2.03	1.32 to 3.09	0.001	
Process location						76.43 (<0.000001)
Prewash	106 (70.7)	150	1.00	NA	Reference	
Postwash	71 (47.3)	150	0.37	0.23 to 0.60	<0.001	
Chilled	60 (25.5)	235	0.14	0.09 to 0.22	<0.001	
APC class (three class)						73.07 (<0.000001)
Class 1	38 (21.4)	178	1.00	NA	Reference	
Class 2	125 (49.0)	255	3.54	2.29 to 5.47	<0.001	
Class 3	74 (72.6)	102	9.74	5.54 to 17.11	<0.001	
APC class (four class)						73.37 (<0.000001)
Class 1	38 (21.4)	178	1.00	NA	Reference	
Class 2	125 (49.0)	255	3.54	2.29 to 5.47	<0.001	
Class 3	67 (71.3)	94	9.14	5.54 to 16.21	<0.001	
Class 4	7 (87.5)	8	25.79	3.08 to 216.10	0.003	
Log APC/cm^2	NA	535	4.34	3.18 to 5.93	<0.001	NA

[a] NA, not applicable.

TABLE 3b. *Model I: Multivariate logistic regression model for outcome of* E. coli *isolation from 535 beef carcasses with APC categorized as 3 levels*[a]

Independent variable	Comparison	Beta	Standard error (beta)	Odds ratio	Odds ratio with 95% confidence intervals	Wald's test *P* value
Plant source	A (fed cattle)	NA [b]	NA	1.00	NA	Reference
	B (cow/bull)	0.625	0.436	1.87	0.80 to 4.39	0.151
	C (cow/bull)	1.257	0.277	3.52	2.04 to 6.06	<0.001
Carcass location	Prewash	NA	NA	1.00	NA	Reference
	Postwash	−0.937	0.264	0.39	0.23 to 0.66	<0.001
	Chilled	−1.892	0.393	0.15	0.07 to 0.33	<0.001
APC class (three class)	Class 1	NA	NA	1.00	NA	Reference
	Class 2	0.540	0.269	1.72	1.01 to 2.91	0.045
	Class 3	1.246	0.341	3.48	1.78 to 6.79	<0.001
Constant	NA	−0.277	0.308	0.76	0.41 to 1.39	0.369

[a] Hosmer and Lemeshow goodness of fit chi-square = 8.261 (8 df; $P = 0.41$).
[b] NA, not applicable.

TABLE 3c. *Model II: Multivariate logistic regression model for outcome of* E. coli *isolation from 535 beef carcasses with APC considered as a log-transformed continuous variable*[a]

Independent variable	Comparison	Beta	Standard error (beta)	Odds ratio	Odds ratio with 95% confidence intervals	Wald's test *P* value
Plant source	A (fed cattle)	NA [b]	NA	1.00	NA	Reference
	B (cow/bull)	0.655	0.439	1.93	0.81 to 4.55	0.136
	C (cow/bull)	1.141	0.281	3.13	1.81 to 5.43	0.001
Carcass location	Prewash	NA	NA	1.00	NA	Reference
	Postwash	−0.858	0.266	0.420	0.25 to 0.71	0.001
	Chilled	−1.595	0.404	0.200	0.09 to 0.45	0.001
Log APC/cm^2	Per log increase in APC	0.979	0.187	2.66	1.84 to 3.84	0.001
Constant	NA	−2.185	0.535	0.11	0.04 to 0.32	0.001

[a] Hosmer and Lemeshow goodness of fit chi-square = 13.97 (8 df; $P = 0.08$).
[b] NA, not applicable.

as the APC increased (Table 3b and 3c). The Hosmer and Lemeshow C statistics for Model I and Model II were nonsignificant, indicating good fit of the model with the observed data in both cases.

DISCUSSION

A critical distinction must be pointed out before judging the potential usefulness of the relationship analyzed in this paper; the linear correlation (r) between the numbers of biotype 1 *E. coli* versus the mesophilic APC (both continuous variables) was not what was examined in the current analysis. That relationship was previously demonstrated to be weak, or nonexistent, when microbial data from beef carcasses were analyzed by a linear regression model to test correlations *(11, 12)*. The hypothesis purported in this paper relates the level of the APC to the frequency of isolation or obtaining a positive *E. coli* sample. This is an association between a categorical variable (APC class) and a discrete or dichotomous variable (i.e., presence or absence of *E. coli*).

In this current work, no attempt was made to demonstrate how changes in carcass decontamination processes would affect a relationship between APC of a carcass and biotype 1 *E. coli*.

Since these samples were collected from various stages of the carcass dressing process as well as different plants, they represent different carcass conditions. Despite these differences, the distribution of samples into APC classes is similar to that reported earlier by the United States Department of Agriculture, Food Safety and Inspection Service *(1)*. In that study, samples classifiable as classes 1 to 4 amounted to 22, 47, 23, and 7% of the total dataset (n = 2,089 carcasses) versus 33, 48, 18, and 1% in the current dataset (n = 535). When APC was specified as a continuous explanatory variable in multiple logistic regression (Table 3c), the OR indicated a 2.66 times increase in the likelihood of *E. coli* isolation from the beef carcass per log increase in APC. Similarly, when APC was treated as a three-level categorical variable, carcasses with APC class 2 and class 3 were 1.7 and 3.5 times more likely, respectively, to contain *E. coli* as compared to APC class 1.

We chose logistic regression as our multivariate analytic method in this report since this technique permitted statistical adjustment for the effects of plant source and carcass location on the relationship between APC and *E. coli* isolation. By looking at the data as a whole, this method also minimized loss of statistical power that would have occurred if the data had been analyzed by cross-stratification.

We found of interest the fact that plant source and carcass location had significant adjusted influences on *E. coli* isolation from beef carcasses. The plant source effect suggests possible variability in plant hygiene, animal source, or carcass characteristics, which are either plant specific or related to seasonality or weather conditions that affect the conditions of the live animal. The decreasing likelihood of *E. coli* isolation as carcasses moved from prewash to postwash to chilled locations (Table 3b and 3c) indicates the carcass processing procedures used at these plants reduces *E. coli* contamination. For example, postwash and chilled carcass sponge samples were 2.5 (i.e., 1 to 0.39) and 6.7 (i.e., 1 to 0.15) times more likely, respectively, to be *E. coli* negative than sponge samples from prewash carcasses (Table 3b).

It is generally accepted that the usefulness of the APC is solely as a measure of original microbial quality and the subsequent shelf life of foods *(15)*. Previously, Miskimin et al. *(17)* presented an analysis of relationships between indicator bacteria and specific pathogens or other groups (including coliforms, *E. coli*, salmonellae, *Staphylococcus aureus*, and *Clostridium perfringens*) in both raw and ready-to-eat foods. That study showed that as APC increased more samples were positive for the presence of *E. coli*. Specifically, as the APC approached the level of 5×10^4 to 1×10^5 CFU/g in raw foods, the rate of *E. coli*–positive samples rose to between 80 and 90% *(17)*. Using the geometric mean of the APC and pathogen numbers of each classification to obtain continuous variables, these authors performed a linear regression analysis that indicated a very low level of correlation (r) between the means of the classifications *(17)*. A lack of linear correlation in this case is not surprising considering that the selected organisms have no real biological relationship to the indicators, other than a potential common conduit.

The qualities of an "ideal" fecal indicator have been discussed *(2, 4, 15)*. The usefulness of indicator organisms must also consider the type and the amount of microflora normally present on or in a food. Because of the great variety of food and food ingredient microbial profiles, there is no generalized relationship that can be made between all potential fecal indicators and the incidence of pathogens for all foods (raw or ready to eat). For instance, fermented foods have a high and desirable microbial load, whereas the surface of a beef carcass immediately after hide pulling is practically sterile for moments after removal. This extreme comparative serves to illustrate the lack of usefulness of the APC for indicating even microbial quality of fermented products. Conversely, since the source of major microbial contamination on a beef carcass is fecal in origin (whether the form of contamination on a carcass is soil on the hide, hoof soil, or dust), one could expect any significant increase in the APC of the surface of that carcass to probably be the result of fecal contamination and a concomitant increase in the isolation rate of *E. coli*, although one would not expect a linear correlation between the APC and the continuous variable of *E. coli* counts *(11, 12)*.

Although the data presented in this analysis include samples that were taken from different major points in the line of processing, nothing is known about the contributions of specific antimicrobial interventions, such as trimming, steam vacuuming, steam pasteurization, and organic acid washes, to the association between the APC and frequency of isolating *E. coli* biotype 1. The contribution of deboning to the ultimate microbial contamination of the product is considered to be significant *(5)*. Such a study might be beneficial to further ascertain the strength of the association of APC class to the rate of isolation of *E. coli* at that point in processing.

Using the APC class level as a gauge, the processor could objectively determine which samples are more likely

to be *E. coli* positive when analysis by the required and more time-consuming quantitative biotype 1 *E. coli* assay is performed *(9)*. For monitoring the ongoing process, an accurate and precise APC value is not necessary for determining the likelihood of *E. coli* contamination after the washing step; rather, classifying the APC into broad groups appears to be adequate. Although the APC is not a direct indicator of the ultimate safety of the resulting raw meat, means do exist to determine the APC class or level of microbial contamination of a carcass surface area in near real time *(3, 13, 19, 20)*. These data could determine the likelihood of *E. coli* contamination, at least during the postwash, prefabrication stages of processing.

This information could benefit beef processors by allowing directed and proactive, rather than reactive measures, to bring the process back into compliance with limits predetermined by the processor.

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