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Evaluation of swab and wash-based sampling methods for process hygiene monitoring of rabbit carcasses

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Abstract

Rabbit meat is increasingly consumed as a nutritious alternative to conventional meats; however, information on appropriate microbiological sampling methods for rabbit carcasses remains limited.

The primary objective of this study was to compare swab- and wash-based sampling methods for microbial monitoring of rabbit carcasses. As a secondary objective, we assessed whether anatomical sampling location (neck vs. hind leg) influenced aerobic plate count (APC) recovery. Carcasses were sampled at a commercial slaughter facility using three methods: surface swabbing of the neck and hind leg, site-specific wash-water sampling of the neck and hind leg, and whole-carcass wash sampling. APC was enumerated using 3M Petrifilm Aerobic Count plates and expressed as log CFU/mL. Within both the surface-swab and site-specific wash-water sampling methods, APC did not differ significantly between neck and hind leg samples, indicating that sampling location had little effect on bacterial recovery. Whole-carcass wash sampling yielded a significantly higher overall APC than combined neck-and-hind-leg swabbing (2.75 ± 0.16 vs. 1.71 ± 0.84 log CFU/mL, respectively; $p < 0.05$), suggesting that whole-carcass wash sampling is more sensitive for detecting surface microbial contamination and may therefore represent a useful approach for routine process hygiene monitoring in rabbit slaughter facilities.

Introduction

Rabbit meat is increasingly being consumed as a nutritious alternative to other meats because of its low fat and cholesterol content, favorable fatty acid profile, and high levels of digestible protein, B vitamins, and essential minerals (Dalle Zotte, 2002; Zamaratskaia *et al.*, 2023). However, rabbit carcasses may become contaminated during slaughter and processing, and microorganisms of public health and hygienic relevance have been reported in rabbit meat and on rabbit carcasses, including *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Listeria monocytogenes* (Kohler *et al.*, 2008; da Silva Guedes *et al.*, 2024). Meat and meat products are among the food categories most frequently associated with foodborne outbreaks worldwide; nevertheless, epidemiological and microbiological data specific to rabbit meat are more limited than those available for poultry, pork, and beef (EFSA, ECDC 2023). Recent surveys have detected antimicrobial-resistant bacteria in rabbit meat, including methicillin-resistant *S. aureus* and extended-spectrum β -lactamase-producing *E. coli*, underscoring the importance of effective microbial monitoring of rabbit carcasses within the One Health framework (da Silva Guedes *et al.*, 2024; Knysz *et al.*, 2025).

Reliable carcass monitoring depends strongly on the sampling method. In red meat species, excision sampling generally recovers higher bacterial counts than nondestructive methods such as swabbing, although swabbing is often preferred operationally because it is practical (Dorsa *et al.*, 1996; Pepperell *et al.*, 2005; Martínez *et al.*, 2010; Gallina *et al.*, 2015). Wet-dry swabbing of defined neck and leg areas has been used in a scientific study to monitor foodborne pathogens and assess microbiological contamination of rabbit carcasses (Kohler *et al.*, 2008). In contrast, whole-carcass wash methods are widely used for poultry carcasses and can provide improved bacterial recovery, particularly for small carcasses (USDA FSIS, 2024). However, comparative data evaluating sampling methods for microbial monitoring of rabbit carcasses are limited.

Accordingly, the primary objective of this study was to compare swab- and wash-based sampling methods for microbial monitoring of rabbit carcasses. A secondary objective was to determine whether anatomical sampling location (neck versus hind leg) affected aerobic bacterial recovery.

Materials and Methods

Sample collection

Rabbit carcasses were sampled from July to September 2020, at a commercial rabbit slaughter facility, in Jeollabuk-do, South Korea. Sampling was conducted after the carcasses had passed through the chilling tunnel and before their transfer to chilled storage. For the anatomical-site comparisons, paired

neck and hind leg samples were collected from the same carcass. Fifteen carcasses were sampled by surface swabbing, and a separate set of 15 carcasses was sampled by site-specific wash-water sampling. The sampling-method comparison was conducted in two independent trials on separate slaughter days. Within each trial, carcasses assigned to the different sampling methods originated from the same slaughter batch, defined as rabbits processed consecutively during the same slaughter run under the same operating conditions. In each trial, three carcasses were sampled per method (3 carcasses per method per trial), with different carcasses assigned to each sampling method.

Sampling procedures

Three sampling methods were evaluated: (i) surface swabbing of the neck and hind leg, (ii) site-specific wash-water sampling of the neck and hind leg, and (iii) whole-carcass wash sampling.

Surface swab sampling

Surface samples were collected from the neck and hind leg areas using a 3M Pipette Swab Plus kit (3M, St. Paul, MN, USA) following the manufacturer's instructions. A defined surface area (10×10 cm) was swabbed at each site using a standardized swabbing technique (MFDS, 2022). After sampling, swabs were returned to the supplied diluent tube and vortexed for 30 s at maximum speed to release microorganisms into the suspension. Swab eluates were serially diluted tenfold in phosphate-buffered saline (PBS; Oxoid, Thermo Fisher Scientific, Basingstoke, UK) from 10^{-1} to 10^{-5} for subsequent enumeration. For combined-site analysis, neck and hind leg suspensions from the same carcass were pooled before dilution.

Wash-water sampling of excised neck and hind leg portions

The entire neck or hind leg portion was aseptically excised and weighed. Each excised portion was placed separately in a sterile plastic bag, and phosphate-buffered saline (PBS) was added at 9 mL per gram of tissue (tissue:PBS = 1:9, w/v). Each bag was manually shaken vertically and horizontally for 60 s to dislodge microorganisms into the wash solution. The resulting wash-water suspension was collected, vortexed for 15 s to ensure homogenization, and serially diluted tenfold in PBS (10^{-1} – 10^{-5}) for microbiological analysis.

Whole-carcass wash sampling

Each carcass was weighed, placed in a sterile plastic bag, and PBS was added at 9 mL per gram of carcass weight (equivalent to a 1:9 sample:diluent ratio). The bag was manually shaken vertically and horizontally for 60 s to ensure uniform distribution of microorganisms into the wash solution. The resulting wash suspensions were serially diluted tenfold in PBS (10^{-1} – 10^{-5}).

Enumeration of APCs

APCs were enumerated using 3M™ Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN, USA) according to AOAC Official Method 990.12, as specified in the manufacturer's instructions. Sample suspensions, including surface-swab eluates, site-specific neck or hind-leg wash-water samples, and whole-carcass wash samples, were serially diluted tenfold in PBS (10^{-1} – 10^{-5}). For each specimen, 1-mL aliquots of the appropriate dilutions were inoculated onto Petrifilm Aerobic Count Plates in technical triplicate (three plates per dilution). The inoculated plates were incubated at $35\pm1^{\circ}\text{C}$ for 48 ± 3 h. All red colonies, regardless of size or color intensity, were counted, and counts from the technical triplicates were averaged to obtain a single APC value per specimen. Samples yielding no colonies were recorded as not detected (ND). For comparability among sampling methods, APC results from swab eluates, site-specific wash-water samples, and whole-carcass wash samples were expressed on a volume basis as log

CFU/mL. Although the swabbed area was standardized to 10×10 cm (100 cm²), conversion to area-based units, such as log CFU/cm², was not performed because differences in microbial recovery and release efficiency between swabbing and washing procedures would introduce additional assumptions into cross-method comparisons.

Statistical analysis

Statistical analysis was conducted using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). APCs were expressed as log CFU/mL. Because neck and hind leg samples were obtained from the same carcasses, detection frequencies were compared using the exact McNemar test. Quantitative APC values were compared using the two-sided exact Wilcoxon signed-rank test; only complete carcass pairs with detectable APC values at both sites were included, and ND values were excluded. Summary statistics (mean, standard deviation [SD], and median) were calculated using detected values only. The effects of sampling method and trial were assessed using two-way analysis of variance (ANOVA), with sampling method, trial, and their interaction as fixed effects. Within each trial, differences among sampling methods were assessed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test. Normality of model residuals was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using the Brown–Forsythe test. Statistical significance was defined as $p < 0.05$.

Results

APCs recovered from wash-water samples collected from the hind leg and neck did not differ significantly. Mean APC values among detected samples were 2.60 ± 1.31 log CFU/mL for the hind leg and 2.88 ± 1.09 log CFU/mL for the neck. Paired analysis of the 11 carcasses with detectable APC values at both sites showed no difference between sites (exact Wilcoxon signed-rank test, $p = 0.831$; Table 1). Detection frequencies were also similar between sites (exact McNemar test, $p = 0.625$; Table 1). Similarly, hind leg and neck swab samples did not differ in detection frequency (exact McNemar test, $p = 0.453$; Table 2) or quantitative APC values among the eight complete detected pairs (exact Wilcoxon signed-rank test, $p = 0.195$; Table 2). These findings indicate that anatomical sampling location (neck versus hind leg) had little effect on APC recovery for either wash-water or swab-based sampling.

In contrast to sampling location, the sampling method had a pronounced effect on APC recovery. The assumptions of the factorial ANOVA were supported: model residuals did not deviate from normality (Shapiro–Wilk test, $p = 0.772$), and no heterogeneity of variance was detected (Brown–Forsythe test, $p = 0.644$). Across the two independent trials, two-way ANOVA showed significant effects of sampling method ($p = 0.0008$) and trial ($p = 0.0016$), whereas the sampling method × trial interaction was not significant ($p = 0.2703$). Whole-carcass wash sampling yielded the highest mean APC in both trials (Table 3).

For the within-trial ANOVA models, neither the residual normality nor the homogeneity-of-variance assumption was rejected (all $p > 0.05$). Within Trial 2, Tukey's HSD showed that whole-carcass wash recovered a substantially higher APC than all swab-based methods (Table 3), whereas APC was similar across sampling methods in Trial 1. However, under commercial abattoir conditions, applying a whole-carcass wash approach can be impractical for rabbits because the carcass size requires a proportionally large volume of diluent and substantial handling capacity. From a feasibility standpoint, hind leg swabbing may serve as a practical alternative. Hind leg swabbing produced an intermediate overall APC (superscript “ab”), suggesting that it can recover higher bacterial counts than neck swabbing alone while remaining operationally more manageable than whole-carcass washing (Table 3).

Discussion

The absence of a strong site effect is consistent with previous reports of comparable bacterial counts between neck and leg sites in rabbit carcasses (Kohler *et al.*, 2008) and may reflect the relatively

heterogeneous distribution of surface contamination following multiple processing steps (Pepperell *et al.*, 2005; Martínez *et al.*, 2010), supporting the suitability of either anatomical site for routine hygiene monitoring.

These findings reinforce the principle that nondestructive swabbing recovers only a fraction of the microorganisms present on carcass surfaces compared with excision- or wash-based methods (Dorsa *et al.*, 1996; Pepperell *et al.*, 2005; Martínez *et al.*, 2010). Swabbing efficiency is influenced by factors such as surface texture, swab material, operator technique, and incomplete release of bacteria into the diluent, all of which contribute to lower and more variable recovery (Pepperell *et al.*, 2005). Although swabbing is attractive for routine monitoring because it is nondestructive and easy to apply, the present data indicate that it may underestimate microbial loads on rabbit carcasses.

Whole-carcass wash sampling provides contact with a substantially larger surface area and facilitates the detachment of loosely attached microorganisms, resulting in higher and more consistent APC recovery. This is consistent with the superiority of whole-carcass wash over swabbing for small carcasses such as poultry (USDA FSIS, 2024), and supports its applicability for rabbit carcass hygiene assessment, given the comparably small size.

The absence of statistical differences between sampling sites suggests that sampling location can be selected based on operational convenience, whereas the choice of sampling method is the critical determinant of microbial recovery. For process hygiene monitoring programs aimed at trend analysis or verification of sanitation effectiveness, whole-carcass wash sampling may provide a more sensitive and representative assessment of microbial contamination on rabbit carcasses than surface swabbing alone.

However, this study has some limitations. The number of carcasses sampled per method per trial was small, and only APCs were assessed. Therefore, the results should be interpreted with caution. Future studies should incorporate larger sample sizes and include coliforms and Enterobacteriaceae as process-hygiene indicators, *E. coli* as a more specific indicator of fecal contamination, and relevant foodborne pathogens such as *Salmonella* spp. and *L. monocytogenes* to evaluate the suitability of the sampling methods for detecting both general hygiene failures and specific public health hazards.

ConclusionS

Sampling method has a greater impact on aerobic bacterial recovery from rabbit carcasses than the anatomical sampling location. No statistical differences in APCs were observed between neck and hind leg samples, indicating that either site is suitable for routine sampling. Whole-carcass wash sampling consistently yielded higher APCs than swabbing methods, suggesting greater bacterial recovery for detecting surface microbial contamination. These findings emphasize the importance of selecting an appropriate sampling method when designing microbial monitoring programs for rabbit slaughter operations and suggest the potential utility of whole-carcass wash sampling as a practical and effective approach for assessing process hygiene in rabbit carcass production.

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Table 1. Comparison of aerobic plate counts between hind leg and neck wash-water samples (not detected values excluded).

Group (total samples, n)	Detected/Total (%)	APC mean $\pm$ SD† (log CFU/mL)	APC median (log CFU/mL)	Detection p (exact McNemar)§	Paired APC p (exact Wilcoxon)‡
Hind leg wash-water (15)	12/15 (80)	2.60 $\pm$ 1.31	2.27	0.625	0.831
Neck wash-water (15)	14/15 (93)	2.88 $\pm$ 1.09	2.86		

† Mean, SD, and median were calculated using detected values only; unit: log CFU/mL. Detection frequencies are shown as Detected/Total (%). ‡ The two-sided exact Wilcoxon signed-rank test compared paired APC values from the same carcasses using complete pairs with detectable values at both sites (n=11); ND values were excluded. § The exact McNemar test compared paired detection statuses between sites. Statistical significance was defined as p<0.05.

Table 2. Comparison of aerobic plate counts (APC) between hind leg and neck swab samples (not detected values excluded). Swabbed area was standardized to 10×10 cm (100 cm²), and APCs are reported as log CFU/mL to maintain a common unit across sampling methods for direct comparison.

Group (total samples, n)	Detected/Total (%)	APC mean $\pm$ SD† (log CFU/mL)	APC median (log CFU/mL)	Detection p (exact McNemar)§	Paired APC p (exact Wilcoxon)‡
Hind leg swab (15)	13/15 (87)	2.51 $\pm$ 0.84	2.67	0.453	0.195
Neck swab (15)	10/15 (67)	1.94 $\pm$ 1.04	1.77		

† Mean, SD, and median were calculated using detected values only; unit: log CFU/mL. Detection frequencies are shown as Detected/Total (%). ‡ The two-sided exact Wilcoxon signed-rank test compared paired APC values from the same carcasses using complete pairs with detectable values at both sites (n = 8); ND values were excluded. § The exact McNemar test compared paired detection statuses between sites. Statistical significance was defined as p<0.05.

Table 3. Effect of sampling method on aerobic plate counts in two trials of rabbit carcasses.

Sampling method	Trial 1 mean $\pm$ SD† (log CFU/mL)	Trial 2 mean $\pm$ SD† (log CFU/mL)	Overall mean $\pm$ SD† (log CFU/mL)
Neck swab	1.74 $\pm$ 0.48 ^a	1.13 $\pm$ 0.40 ^b	1.43 $\pm$ 0.52 ^b
Hind leg swab	2.39 $\pm$ 0.44 ^a	1.44 $\pm$ 0.48 ^b	1.92 $\pm$ 0.65 ^{ab}
Neck + hind leg swab	2.26 $\pm$ 0.89 ^a	1.15 $\pm$ 0.13 ^b	1.71 $\pm$ 0.84 ^b
Whole-carcass wash	2.81 $\pm$ 0.17 ^a	2.69 $\pm$ 0.16 ^a	2.75 $\pm$ 0.16 ^a

† Values are mean $\pm$ SD; n = 3 carcasses per method per trial and n = 6 per method overall. Within each column, different superscript letters indicate significant differences (p < 0.05; Tukey's HSD test). Statistical significance was defined as p<0.05