COMPARISON OF EXCISION, SWABBING AND RINSING SAMPLING METHODS TO DETERMINE THE MICROBIOLOGICAL QUALITY OF BROILER CARCASSES

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ABSTRACT

Skin excision, swabbing with cotton wool and whole carcass rinse are three common sampling methods of poultry carcasses. The objective of this study was to compare the three different sampling methods for enumeration and monitoring of bacteria on broiler carcasses. Total viable counts, *Pseudomonas* spp., *lactic acid bacteria*, *Brochothrix thermosphacta* and Enterobacteriaceae recovered by each sampling method were enumerated using the pour plate technique. Rinsing and excision recovered a similar level ($P > 0.05$) of the total viable counts, whereas swabbing yielded a lower level ($P < 0.05$). For *Pseudomonas* spp., *lactic acid bacteria* and *B. thermosphacta*, rinsing recovered the highest counts, followed by excision and finally the swabbing. There was no significant difference ($P > 0.05$) to detect Enterobacteriaceae by the three methods. Polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) was used to monitor bacterial constituents. Compared with rinsing, the dice coefficient was 69.2% for excision and 32.3% for swabbing. The results revealed that great differences existed among the sensitivity of microorganism detection by the three methods, rinsing > excision > swabbing. Considering the bacterial recovery and DGGE profile, rinsing seems to be the preferable sampling method for enumeration and monitoring of bacteria on broiler carcasses whereas swabbing is poor.

PRACTICAL APPLICATIONS

This work compared the efficiency of three sampling methods (excision, swabbing and rinsing) to evaluate bacteria on broilers using culture-dependent and culture-independent methods. The results indicate that whole carcass rinse would be a preferable sampling method to monitor the bacteria on broiler carcasses, especially using the culture-independent method.

INTRODUCTION

Microbiological criteria have become an important source of information in developing hazard analysis critical control point and quality management systems of poultry slaughter plants (Brown *et al.* 2000). Sampling methods are necessary for critical control point determination and overall improvements in the microbiological quality of commercially processed broiler carcasses. Various sampling methods are available to determine microbiological quality of carcasses. There are three principal methods for sampling poultry carcasses, skin excision, swabbing with cotton wool and whole carcass rinse (Palumbo *et al.* 1999; Gill and Badoni 2005). Rinse sampling is used in the U.S.A. within the United States Department of Agriculture Food Safety and Inspection Service pathogen reduction program, whereas sampling of skin is preferred in the European Union. The relative efficacy of several commonly used sampling techniques for bacterial detection on poultry carcasses has been evaluated (Sarlin *et al.* 1998; Jørgensen *et al.* 2002; Mead *et al.* 2010). However, application of the rinsing method on the microorganism detection, especially spoilage bacteria, has received little attention.

Traditionally, the spoilage bacteria on carcasses are analyzed by culture-dependent methods. Currently, molecular methods have become a valid support to traditional techniques. Applied molecular microbiology is a fast-moving area. One of the branches of this discipline is involved in the development of molecular methods for the identification and monitoring of microorganisms. Microbial species have the same length of 16S rRNA gene fragments although their DNA sequences differ. Based on this, the polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) technique has been gaining popularity as a versatile tool for investigating microbial diversity (Ercolini 2004). Although the relative efficacies of destructive and various nondestructive sampling methods have been compared (Gill and Jones 2000; Gill and Badoni 2005), few studies evaluated sampling methods for culture-independent analysis of microorganism (Pearce and Bolton 2005).

The present study was undertaken to evaluate the effectiveness of three sampling methods, swabbing, excision and rinsing, for recovery and monitoring of microorganisms of broiler carcasses using culture-dependent and culture-independent technologies.

MATERIALS AND METHODS

Broiler Carcasses

Ninety broiler carcasses (733 to 855 g) were obtained from a local processing plant immediately after evisceration and were placed in sterile containers and transported on ice to the laboratory within 45 min. Before sampling, each carcass was split aseptically along the breast bone and opened to expose the body cavity.

Excision Samples

Broiler skin of 20 cm^2 each were excised from five sites: leg, breast, neck, around the visceral cavity and under the wings (total area 100 cm^2). Each skin was aseptically transferred to a sterile sampling bag containing 100 mL of saline, peptone dilution water (SPW, containing 0.85% NaCl and 0.1% peptone). The contents of the bag were pummeled for 2 min in a stomacher.

Swab Samples

A sterile cotton wool that had been moistened with SPW was used to swab an area of approximately 20 cm^2 at each of the five sites that were equivalent to those sampled by excision: leg, breast, neck, around the visceral cavity and under the wings (total area 100 cm^2). The delimited area was then swabbed with, second, a dry cotton wool. The cotton wool was pummeled with 100 mL of peptone water for 2 min using a stomacher.

Rinse Samples

The whole carcasses were transferred to a sterile sampling bag with 300 mL of SPW and shook for 2 min. The surface areas of samples were determined according to Gill and Badoni (2005). Carcasses were split along the breast bone and opened to expose the body cavity. Split carcass arranged for measurement of the distances between the base of the neck (a) and the base of the uropygium (b), and between the caudal ends of the divided breast bone (cd), with the surface area of the body cavity being estimated as ab × cd. The skin was stripped from the rest of the carcass and was arranged in an approximately rectangular form without substantial stretching. The area of the outer surface of each carcass was calculated as the area of the excised skin plus the area obtained by multiplying the mean length of the two wings by the mean of their maximum circumferences. The total surface area was calculated by adding to that value the area obtained by multiplying the lengths measured along and across the exposed body cavity (Gill and Badoni 2005). For the cm^2/mL conversion factor, divide total cm^2 by 300 mL of SPW used for the carcass rinse.

Microbiological Analysis

For determination of bacterial counts, the homogenate was serially diluted in triplicate (1:10) in SPW. Once the dilution was made, 1-mL volumes were prepared for culture using the pour plate technique. For the enumeration of total viable counts (TVC), plate count agar (PCA, Lu Qiao Company, China) was incubated at 37C for 48 h. *Pseudomonas* spp. were enumerated on cetrimide fucidin cephaloridine agar (Oxoid Company, Cambridgeshire, England) and incubated at 25C for 48 h. *Brochothrix thermosphacta* were counted on streptomycin thallous acetate actidione agar (Oxoid Company) at 30C for 48 h. *Lactic acid bacteria* (LAB) were counted on de Mann–Rogosa–Sharpe agar (Lu Qiao Company) at 30C for 48 h. The white colonies were counted. Violet red bile glucose agar (Lu Qiao Company) were incubated at 37C for 48 h. Large colonies with purple haloes were counted as Enterobacteriaceae.

All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. Microbiological data were transformed into logarithms of the number of colony-forming units (cfu/cm^2). cfu/cm^2 calculation: Total cfu on plates = cfu/mL, cfu/mL ÷ cm^2/mL = cfu/cm^2.

PCR-DGGE Analysis

Direct extraction of genomic DNA from samples at each sampling point was undertaken as follows: 70 mL of homo-

genate was aseptically collected and centrifuged at 2,000 × g at 4C for 10 min (Avanti J-E, Beckman Coulter, CA). The supernatant (about 50 mL) was aseptically transferred into a sterile centrifuge tube and recentrifuged at 12,000 × g at 4C for 10 min. The upper layer was discarded and the pellet was resuspended in 1 mL of sterile distilled water for DNA extraction. Total bacterial DNA was extracted using GenEluteTM Kit (Tiangen Biotech, Beijing, China) following the manufacturers' instructions. Finally, DNA was suspended in 80 μL of TE buffer and stored at −20C.

Primers U968-GC (5′-CGC CCG GGG CGC GCCCCG GGC GGG GCG GGG GCA CGG GGG GAACGC GAA GAA CCT TAC) and L1401 (5′-GCG TGT GTA CAA GAC CC) were used to amplify the V6–V8 regions of the bacterial 16S rDNA. DNA concentration was measured using the Nanodrop 2000 spectrophotometer (Thermo, Chicago, IL). DNA extracts were mixed with TE buffer to obtain the same amount of DNA. PCR reactions were performed in a total volume of 25 μL, and included GoTaq Green Master Mix 12.5 μL (Promega, Fitchburg, WI), 0.5 μL (10 pmol/mL) of each primer, 1 μL of DNA template, and 10.5 μL of ddH_2O. The following PCR program was used: 94C for 5 min, 35 cycles of 94C for 1 min, 56C for 30 s, 72C for 1 min. Finally, the reaction was stopped with an extension step at 72C for 7 min. PCR products (5 μL) were analyzed in 1.2% agarose gel electrophoresis in 0.5 × Tris Ace-tate EDTA (TAE) buffer (50 × TAE stock solution consisted of 2 M Tris base, 1 M glacial acetic acid and 50 mM Ethylene Diamine Tetraacetie Acid [EDTA]).

The PCR products were analyzed by DGGE using a Bio-Rad DCode apparatus (Bio-Rad, Richmond, CA). Electrophoresis was performed in a 0.8-mm-thick polyacrylamide gel (8% [w/v] acrylamide-bisacrylamide [37.5:1]) containing a 35–55% urea-formamide denaturing gradient (100% corresponds to 7 M urea and 40% [w/v] formamide). The gel was subjected to a voltage of 200 V for 10 min and then 85 V for 16 h in 0.5 × TAE buffer. After electrophoresis, gels were stained with ethidium bromide (0.5 mg/L) for 20 min and analyzed under UV illumination using the GelDoc 2000 system (Bio-Rad).

Statistical Analysis

Microbiological data were transformed into logarithms of the number of colony-forming units (cfu/cm^2). Values for the mean (X) and standard deviation (SD) were calculated. Six replicate measurements are presented. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test using the SPSS 16.0 (SPSS Inc., Chicago, IL). Significant differences were determined at the 5% level ($P < 0.05$). The fingerprints of the DGGE profile were analyzed by unweighted pair group method with arithmetic means using Quantity One 1D Analysis software (Bio-Rad).

RESULTS AND DISCUSSION

For rinsing, values for the mean ± the SD for the areas of the outer surfaces of carcasses and areas of the outer plus the inner surfaces of carcasses were 395.6 ± 33.8 cm^2 and 345.7 ± 25.8 cm^2, respectively.

The ability of three sampling methods to recover bacteria is presented in Table 1. The TVC obtained by rinsing and excision was significantly higher ($P < 0.05$) than swabbing. Furthermore, there was no significant difference ($P > 0.05$) between the sets of rinsing and excision. For the three methods, statistical analysis of the recovery data of *Pseudomonas* spp., *B. thermosphacta* and LAB indicated that they were significantly different ($P < 0.05$). The highest counts were recovered using the rinsing method followed by excision, and the lowest counts by the swabbing method. Interestingly, there was no significant difference ($P > 0.05$) in the counts of Enterobacteriaceae among the three groups.

In general, as the log count is likely to be below the log mean, and has an equal chance of being above or below the mean log, the count will usually underestimate the true level of microorganism in food. The degree of underestimation will depend on the variance which is estimated by SD^2 (Kilsby and Pugh 1981). Comparison between different bacteria showed that variance of one sampling method was not constant, but neither was the variance for the same bacteria using

TABLE 1. STATISTICS FOR TVC, *PSEUDOMONAS* SPP., *B. THERMOSPHACTA*, LAB AND ENTEROBACTERIACEAE COUNTS (log cfu/cm^2) RECOVERED BY EXCISION, SWABBING AND RINSING

	TVC		*Pseudomonas* spp.		*B. thermosphacta*		LAB		Enterobacteriaceae	
	X	SD	X	SD	X	SD	X	SD	X	SD
Excision	4.30^A	0.04	3.01^B	0.06	2.94^B	0.31	2.58^B	0.16	4.65^A	0.29
Swabbing	3.79^B	0.29	2.81^C	0.12	2.33^C	0.32	1.62^C	0.47	4.19^A	0.32
Rinsing	4.60^A	0.04	3.40^A	0.07	4.46^A	0.18	3.37^A	0.09	4.57^A	0.23

$^{A–C}$ With the same row and concerning the same flora, results with the same letter are not significantly different ($P > 0.05$).

LAB, lactic acid bacteria; SD, standard deviation; TVC, total viable counts; X, mean of log-transformed bacterial numbers.

different sampling methods (Table 1). For the TVC and *Pseudomonas* spp., the variance in the set of swabbing was higher, while it was similar in the sets of excision and rinsing. In the detection of *B. thermosphacta*, LAB and Enterobacteriaceae, the SD^2 values of the three sampling methods were different: swabbing > excision > rinsing. It means that a larger variability was observed by excision and swabbing compared with rinsing.

The present study showed that recovery of bacteria from a carcass surface was dependent on the sampling method used (Gill and Badoni 2005; Ghafir and Daube 2008). The lowest recovery number and highest variation were achieved by swabbing, suggesting that swabbing was the poorest method. The lower recovery levels of the swabbing method may be attributed to the topography of the skin or the attachment of bacteria to the skin (Ghafir and Daube 2008). In addition, many factors, including swabbing materials and carcass type, may have a significant effect on bacteria recovery by swabbing (Pearce and Bolton 2005). However, swabbing is considered advantageous for the meat industry because it is less laborious than excision and does not compromise meat quality (Lindblad 2007). In general, excision is superior to swabbing, based on the fact that higher numbers are recovered and low variation is achieved. However, Bolton (2003) suggested that swabbing may be more reliable for monitoring microorganisms when it covers larger carcass areas than excision. The findings of the current study are in agreement with Gill and Badoni (2005) who reported that excision and rinsing will recover similar numbers, while swabbing recovered lower. Although rinse technique was sensitive in the ability to detect microorganism, it was considered inconvenient for sampling carcasses. First, swabbing and excision offer practical advantages over the traditional whole carcass rinse, because of the ease and rapidity of collection, reduced enrichment medium required and the possibility of sampling prior to complete evisceration (Sarlin *et al.* 1998; Mead *et al.* 2010). Second, measurement of the surface areas of carcasses is necessary for rinse methods, but it is hardly possible to determine (Jørgensen *et al.* 2002; Gill and Badoni 2005). Many published studies calculated the surface area using various formulas that relate the surface area to the weight of broiler carcasses (Thomas 1978). However, the intensive breeding to which broilers have been subjected could be expected to alter the relationship over time (Thomas 1978; Gill and Badoni 2005).

PCR-DGGE was used in this study to compare the three sampling methods considering it provides an alternative to traditional tools for the identification of dominant species. Quantity and integrity of extracted DNA were assessed by agarose gel electrophoresis (Fig. 1). As was expected, the PCR generated one main amplicon approximately 500 bp in size for the DNA studied. Gel electrophoresis reveals

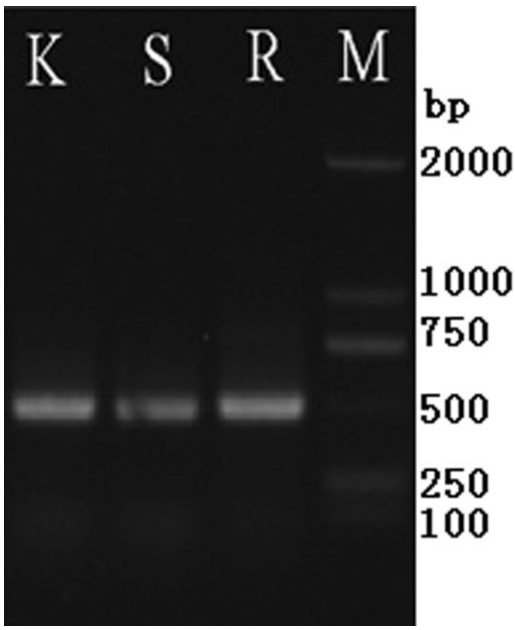

FIG. 1. PCR PRODUCTS OF V6-V8 REGIONS FROM THREE SAMPLING METHODS: M, DNA MARKER (DL2000); K, DNA EXTRACT FROM EXCISION SAMPLES; S, DNA EXTRACT FROM SWABBING SAMPLES; R: DNA EXTRACT FROM RINSING SAMPLES

that bacterial DNA can be efficiently extracted from the homogenate obtained by three microbiological sampling methods. There were no significant differences in the purity and integrity of DNA. However, the DGGE band patterns obtained by three methods were different (Fig. 2a). For bands presented in rinsing, 10 and 12 bands at the top or bottom of gel did not appear in excision and swabbing, respectively. Moreover, intensity of majority bands (bands 9, 11, 12 and 14) in rinsing was similar to that in excision but different from that in swabbing. To compare the similarity of the DGGE band patterns, the bands were quantified with Quantity One software (Fig. 2b). Compared with rinsing, the dice coefficient was 69.2% for excision and 32.3% for swabbing. DGGE band intensity is related to initial DNA template amount in the extracted sample. As DGGE bands theoretically represent different groups and species of bacteria (Ercolini 2004), it is obvious that some bacterial species were missed when using the excision or swabbing methods. A previous study showed that the percent prevalence of microorganism among the carcass sites were different (Vaidya *et al.* 2005). Because of this reason, more sites were sampled by rinsing and more species were detected. Although the same sites were sampled by excision and swabbing methods, bands 8, 11, 15 and 18 only presented in excision. This result supports a previous study (Sarlin *et al.* 1998) that excision was more sensitive to detect microorganism than swabbing. Our research indicated that in order

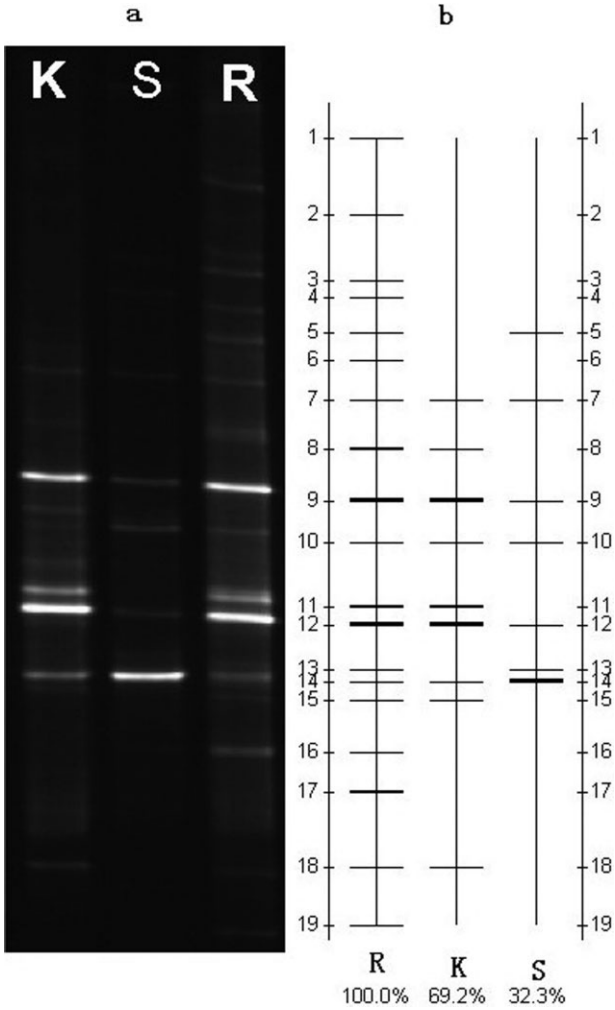

FIG. 2. 35–55% DGGE PROFILES (a) OF PCR PRODUCTS OBTAINED BY EXCISION (K), SWABBING (S) OR RINSING (R) AND ELECTROPHORETIC COMPARISON CHART (b)

to gain more information on bacterial flora of broiler carcasses, rinsing is better than both other sampling methods.

In conclusion, this study shows that rinsing is preferable to recover bacteria considering high mean log number and low SD. PCR-DGGE analysis and band comparison demonstrate that the DNA of the microbial community can be efficiently extracted from the homogenate obtained by three microbiological sampling methods, but the bacterial community structures are significantly different. Considering that higher bacterial recovery and more bands of DGGE profile were obtained by rinsing, we would propose that rinsing is the preferable method for sampling broiler carcasses to monitor microorganisms by DGGE.

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