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Short communication

Comparison of four sampling methods for microbiological quantification on broiler carcasses

Gunvor Elise Nagel Gravning [a,*], Ole-Johan Røtterud [a], Solfrid Bjørkøy [b], Merete Forseth [b], Eystein Skjerve [c], Ann-Katrin Llarena [c], Astrid Lian [d], Gro S. Johannessen [e], Sigrun J. Hauge [a]

[a] Animalia - Norwegian Meat and Poultry Research Center, P.O. Box, 396 Økern, N-0513, Oslo, Norway
[b] Norsk Kylling, Bygget 6, N-7290, Støren, Norway
[c] Norwegian University of Life Sciences, Dept. of Paraclinical Sciences, P. O. Box 369 Sentrum, N-0102, Oslo, Norway
[d] Trondheim Kommune Bydrift Analysesenteret, Landbruksvegen 5, N-7047, Trondheim, Norway
[e] Norwegian Veterinary Institute, P. O. Box 750 Sentrum, N-0106, Oslo, Norway

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ABSTRACT

The aim of this study was to evaluate four different sampling methods for quantification of bacterial contamination of broiler carcasses at slaughter. Various sampling techniques are used worldwide for sampling carcass surfaces and the results are often presented with different units of measurement, such as cfu (or log cfu) per mL, per cm^2, per carcass, or per gram. Sampling was performed in a commercial abattoir with naturally contaminated carcasses (n = 100). Sampling methods compared were: whole-carcass rinse (WCR) in 200 mL liquid, 10 g of neck skin and breast skin, and gauze cloth swabs (3 sites x 100 cm^2). Petrifilms were used for analyses of the samples for total plate count, Enterobacteriaceae, and *E. coli*. The results were converted into log cfu per cm^2.The recoveries of Enterobacteriaceae and *E. coli* were highest from samples collected by WCR, followed by neck-skin excision (recovered 80–100% of WCR), then breast-skin excision (recovered 50–65% of WCR), and finally swabbing (recovered 40–50% of WCR). In conclusion, the WCR sampling method provides the best reflection of the extent of carcass contamination.

1. Introduction

To ensure that high hygienic standards are maintained during the broiler slaughtering process, the meat industry monitors and controls carcass contamination using a Hazard Analysis and Critical Control Points (HACCP) approach, Good Hygienic Practice (GHP), and microbiological testing. However, sampling methods for microbiological testing vary between abattoirs and countries. For monitoring of process hygiene, several sampling methods and indicator bacteria are used. ISO 17604 (ISO, 2015) describes protocols for the most common methods: WCR, neck-skin excision, breast-skin excision, and swabbing. ISO 17604 (ISO, 2015) states that the choice of sampling method for process hygiene depends mainly on the aim of the microbiological examination, the sensitivity required, and practical considerations.

Published studies on broiler slaughter hygiene present the results of microbiological analyses using various units of measurement: log cfu or cfu per mL, per cm^2, per carcass, or per gram. The sampling location along the slaughter line also varies, with sampling of both warm and cold carcasses before and after chilling and the bacterial load varies along the process line (Althaus et al., 2017; Loretz et al., 2010). It is therefore difficult to compare results and the effects of interventions being evaluated in the studies. The relative efficacy of commonly used sampling techniques for bacterial detection on poultry carcasses has been reported in previous studies (Cox et al., 2010; Jørgensen et al., 2002; Simmons et al., 2003; Zhang et al., 2012). Gill and Badoni (2005) state that when poultry carcasses are within a known range of sizes, a single factor can be used to convert from bacterial counts per carcass to counts per unit area of carcass surface. However, as far as we are aware, no universal conversion factors between the results of the various sampling methods have been studied, established, or used. For this reason, the aim of this study was to compare four sampling techniques ability to representatively quantify the microbiological load of broiler carcasses and to assess the relative recovery of microbiological contamination by the four sampling methods. Also, the identification of an acceptable and appropriate sampling technique to be used in a subsequent decontamination study was included in the aim for this study.

* Corresponding author.
E-mail address: elise.gravning@animalia.no (G.E. Nagel Gravning).

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2. Materials and methods

2.1. Carcass sampling

Samples (n = 100) were collected in a commercial Norwegian broiler slaughterhouse on a single day in 2019. Warm carcasses were obtained from two flocks with flock sizes of 12,996 and 13,121 birds, the average slaughter weights for each flock were 1655 g and 1738 g, and the coefficients of variation (CV%) of slaughter weights were 14.2 and 14.1%, respectively. Samples were collected from one location on the slaughter line after the official meat inspection, which was after scalding (55–57 °C), plucking, evisceration, but before washing and chilling. The slaughter line speed was 9000 carcasses per hour. Sampling was performed by four study personnel, working in pairs. Twenty five carcasses were collected for sampling for each of the four sampling methods but the same carcass was used for sampling neck and breast skin (25 for rinsing, 25 for swabbing, 25 for neck skin and breast skin); total 75 carcasses (25 × 3 carcasses) and 100 samples in total.

2.2. Sampling methods

Two non-destructive methods and two destructive methods were used to obtain samples. The non-destructive methods were: the WCR method (Method A) and the gauze-cloth swabbing method (Method D); the two destructive methods were: excision of neck skin (Method B) and of breast skin (Method C). These methods were applied as described in ISO 17604:2015 (ISO, 2015). For Method A, the carcass for sampling was put in a plastic bag by an operator whose hands were encased in plastic bags, and 200 mL sterile peptone water was added. The carcasses were shaken in the bags for 30 s before being removed, and the peptone water transferred to plastic bottles. For Methods B and C, the carcasses were placed on off-line hooks by an operator whose hands were encased in sterile plastic bags. Skin was excised aseptically using a sterile disposable scalpel, and with the other hand, the skin sample was held within an inverted sterile plastic bag, which was then everted such that the sample was placed into the sterile bag. The same carcass was used for sampling with both Method B and Method C. For Method D, a sterile medical-gauze cloth swab (10 × 10 cm) (Mesosoft, Mölnlycke Health Care AB, Sweden) was moistened with 10 mL sterile peptone water. The test area on the breast, back, and around and inside the rectum was then swabbed with the cloth using 10 horizontal and 10 vertical movements (approx. 30 s) representing an area of 300 cm^2. One gauze swab per carcass was placed in a stomacher bag (BagLight PolySilk, Interscience, St Nom, France). All samples were stored at 3–6 °C overnight and then analyzed the following day.

2.3. Microbiological analysis

The samples were analyzed using 3M™ Petrifilm™ (3M Microbiology, St Paul, MN, USA) for total plate count (TPC; Aerobic Count Plates 6400), Enterobacteriaceae (Enterobacteriaceae Count Plates 6420), and *E. coli* (Select *E. coli* Count Plates 6434). Samples from Method A, rinsing of carcasses, were processed by first shaking the bottle by hand, followed by serial dilution of the rinsate. Neck skin samples obtained using Method B were processed by taking 10 g of the sample with a sterile knife, adding 90 mL sterile peptone water, and then homogenizing for 30 s with a stomacher (Laboratory blender, Stomacher 400, Seward, UK). Breast skin samples obtained by Method C followed the same procedure as for Method B. For the swab samples (Method D), to each bag containing one cloth was added 10 mL sterile peptone water, and the bag homogenized, as for the Methods B and C samples, for about 30 s. For each of the Methods, 1 mL of the dilutions was plated on Petrifilm as described by the manufacturer. The Petrifilms for TPC were incubated at 30±1 °C for 72 ± 3 h, the Enterobacteriaceae Petrifilms were incubated at 37±1 °C for 24 ± 2 h, and the *E. coli* Petrifilms were incubated at 42±1 °C for 24 ± 2 h. After incubation, all Petrifilms were read according to the manufacturer's instructions.

2.4. Conversion of unit of measurement to cfu/cm^2

To be able to compare the results from the four sampling methods, the results were converted to log cfu/cm^2. Results from Method A, the WCR were initially presented as cfu/carcass (or 200 mL) and the inner and outer surface areas of an average carcass were estimated for conversion to cfu/cm^2. For the determination of skin surface area, the methodology described by Gill and Badoni (2005) was used. The outer and inner surfaces of carcasses were estimated to be approximately 1150 cm^2 and 150 cm^2, respectively, a total surface area of 1300 cm^2 was applied in the conversion of unit of measurement for cfu/carcass (200 mL) to equivalent cfu/cm^2 for the WCR method (Method A). Methods B and C were initially presented as cfu/g according to the laboratory procedures. To determine the size of surface skin area, 10 g of neck skins (n = 10) and breast skin (n = 10) were measured by a ruler in cm^2. Since the necks usually are cut off before chilling, neck-skin measurements were performed on skin from warm carcasses, and breast-skin measurements were performed on skin from chilled carcasses. The results from measuring the surface area of 10 g of neck and breast skin in cm^2 were 37.5 cm^2 (range 24.5–56.0) and 56.3 cm^2 (range 38.5–71.2), respectively. Results from Method D (swabbing) were initially presented as cfu/cloth (or 300 cm^2). For swabbing, the results were converted to cfu/cm^2 by dividing the results by 300 cm^2, this representing the combined swabbed area of 100 cm^2 on the breast, 100 cm^2 on the back, and 100 cm^2 around and inside the pelvic cavity.

2.5. Statistical analysis

Statistical analyses were conducted in Stata/MP 16.0 (StataCorp, College Station, TX) using ANOVA and regression analyses. The data were transformed from cfu per sample to $\log_{10}$ cfu per cm^2. TPC results below the limit of detection were set to a decimal lower and high, uncountable *E. coli* results were set to a decimal higher.

Descriptive statistics showed some deviation between the mean and the median results of coefficients for the TPC, Enterobacteriaceae and *E. coli* for all four sampling methods. Median regression analyses (non-parametric regression) were performed with TPC, Enterobacteriaceae and *E. coli* as response variables in each model and sampling methods as explanatory variables. Model fit and residuals were checked using graphical techniques. The level of significance was set at $P \leq 0.05$.

3. Results

Median recovery results for the non-destructive methods were for the WCR method (Method A) 4.3 log cfu/cm^2, 4.1 log cfu/cm^2, and 4.1 log cfu/cm^2 for TPC, Enterobacteriaceae and *E. coli*, respectively, and for the swabbing method (Method D), the corresponding median values were 2.2, 1.8, and 1.7 log cfu/cm^2, respectively. For the destructive methods, the median recovery results for the neck-skin method (Method B) were 4.4, 3.4, and 3.4 log cfu/cm^2 for TPC, Enterobacteriaceae, and *E. coli*, respectively; for breast skins (Method C), the corresponding medians were 2.2, 1.9, and 1.9 (Table 1).

Thus, in terms of recovery efficiencies, the method resulting in the highest values for Enterobacteriaceae and *E. coli* was WCR (Method A), followed by Method B (neck skin), Method C (breast skin), and Method D (swabbing) (Fig. 1). Median regression models for response variables TPC, Enterobacteriaceae and *E. coli* confirmed the difference between sampling methods (Table 2). The coefficients quantify the expected change in TPC, Enterobacteriaceae and *E. coli* log cfu/cm^2 for each sampling method, compared with WCR (Method A) as baseline level. For TPC, the neck-skin sampling method (Method B) provided slightly higher results than the WCR (Method A); the regression coefficient was 0.03, with a p-value 0.894. However, for recovery of Enterobacteriaceae and *E. coli*, the WCR provided higher results than all the other methods

Table 1
Mean ± SD (standard deviation), median, minimum and maximum results presented as log cfu/cm^2 for the Total Plate Count (TPC), Enterobacteriaceae and *E. coli* for the whole-carcass rinse (WCR) (A), neck skin (B), breast skin (C) and swabbing (D) methods. Different letters indicate significant differences between methods at P ≤ 0.05 level by ANOVA.

Microorganism	WCR method A	Neck skin method B	Breast skin method C	Swabbing method D
Mean TPC (±SD)	4.4 (0.6) a	4.3 (0.7) a	2.8 (0.7) b	2.3 (0.4) b
Median TPC	4.3	4.4	2.2	2.2
Min TPC	3.4	2.4	2.2	1.8
Max TPC	5.8	5.6	4.4	3.7
Mean Enterobacteriaceae (±SD)	4.0 (0.7) r	3.4 (0.6) s	2.2 (0.6) t	1.9 (0.5) t
Median Enterobacteriaceae	4.1	3.4	1.9	1.8
Min Enterobacteriaceae	2.7	2.2	1.4	0.8
Max Enterobacteriaceae	5.5	4.9	3.8	3.4
Mean *E. coli* (±SD)	3.95 (0.7) x	3.40 (0.6) y	2.2 (0.6) z	1.8 (0.6) z
Median *E. coli*	4.1	3.4	1.9	1.7
Min *E. coli*	2.1	2.2	1.4	0.8
Max *E. coli*	5.3	4.7	3.8	3.6

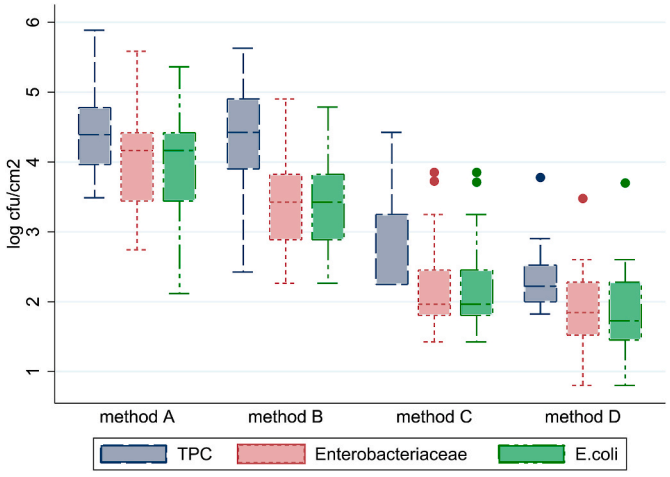

Fig. 1. Box-plot results for Total Plate Count (TPC), Enterobacteriaceae, and E. coli log cfu per cm^2 (n = 25 for each method) for sampling method A) whole-carcass rinse, method B) neck-skin excision, method C) breast-skin excision, and method D) swabbing. On each box, the central mark indicates the median, the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively, and the whiskers extend to the most extreme data points not considered outliers; the outliers are plotted individually using the dot symbol.

(P < 0.05). All three regression models produced results with a reasonable fit, as measured by pseudo R^2 statistics (0.44–0.51). Thus, ranking the methods produced the following overall result: WCR > neck skin > breast skin > swabbing. Compared with WCR in log-units, neck skin excision recovered more than 95% of TPC and 80–85% of Enterobacteriaceae and *E. coli*. Correspondingly, breast skin excision recovered about 50–65% of TPC and 45–55% of Enterobacteriaceae and *E. coli,* and swabbing recovered 50–55% of TPC and 40–50% of Enterobacteriaceae and *E. coli* compared to WCR results. The variance between samples for the two best methods (WCR, Method A, and neck skin, Method B) was lower for the rinsing method than for the neck-skin sampling. The coefficients of variance (CV) for the neck-skin sampling method (Method B) were 0.18 for TPC and 0.20 for both Enterobacteriaceae and *E. coli*. For the rinsing method (Method A), the CV were 0.14 for TPC and 0.18 for both Enterobacteriaceae and *E. coli*.

4. Discussion

Our results showed that the WCR method produced a significantly higher recovery of Enterobacteriaceae and *E. coli* (P < 0.05) compared with the other sampling methods tried (neck skin, breast skin, and swabbing). However, the TPC level was approximately the same for the WCR method and the neck-skin excision method. Swabbing and breast-skin excision produced the lowest recovery results.

The initial results from the lab were transformed into the same unit of measurement (bacteria per cm^2) to enable simple comparison of methods. One challenge with using this sort of transformation is that distortions may occur, as it was necessary to estimate and measure different surface areas of an average broiler: the whole surface area both outside of the skin and inside the cavity; the area of 10 g of neck and the area of 10 g breast skin. Determining the surface area of carcasses is difficult because of their shape and the elasticity of poultry skin. Furthermore, the breast-skin measurements were on chilled samples, whereas for neck skins measurements the skins were warm and thus maybe more elastic. However, as the bacterial numbers were presented as log values, this level of accuracy for surface-area estimation is probably sufficient (Brown et al., 2000), and the numbers obtained by the four sampling methods could be compared. Other studies have reported similar ranking of sampling methods and have achieved similar results (Gill & Badoni, 2005; Zhang et al., 2012). Although various formulae relating the surface area to the weight of broiler carcasses have been published, differences between breeds, growth rates, and live weights of broiler chickens, can alter this relationship (Gill & Badoni, 2005). In our study, the sampled carcasses were not weighed, but instead we used the mean slaughter weight for the two flocks in the study to estimate the surface area of an average carcass.

Several studies have indicated that destructive methods, with stomaching or blending of surface tissues, are the most effective sampling methods because they provide more reliable and less variable bacterial counts than other sampling techniques and there is almost complete recovery of those bacteria that are firmly attached (Capita et al., 2004, pp. 1303–1308; Dorsa et al., 1996; Fliss et al., 1991; Gill & Jones, 2000; Nortje et al., 1982; Werlein, 2001). However, in our study

Table 2
Median regression analyses (non-parametric regression) with TPC, Enterobacteriaceae and *E. coli* as response variables in each model and sampling methods as explanatory variables. The whole-carcass rinse (WCR) is baseline and the other three methods; neck skin, breast skin and swabbing, are compared and presented with respective median regression coefficients for Total Plate Count, Enterobacteriaceae and *E. coli*.

Sampling	TPC		Enterobacteriaceae		*E. coli*	
	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value
Intercept	4.39 (4.03/4.74)	0.00	4.16 (3.84/4.48)	0.00	4.16 (3.83/4.49)	0.00
WCR method	0.0	–	0.0	–	0.0	–
Neck skin method	0.03 (−0.46/0.53)	0.89	−0.74 (−1.18/-0.29)	0,00	−0.74 (−1.21/-2.68)	0.00
Breast skin method	−2.14 (−2.64/-1.64)	0.00	−2.20 (−2.64/-1.75)	0,00	−2.20 (−2.67/-1.73)	0.00
Swabbing method	−2.16 (−2.66/-1.67)	0.00	−2.31 (−2.76/-1.87)	0.00	−2.43 (−2.90/-1.96)	0.00

we obtained higher recoveries of *Enterobacteriacae* and *E. coli* using the rinsing method than the destructive methods when we converted our results into the same unit of measurement, cfu per cm^2. Hutchison et al. (2006) claimed that there can be more variation between samples with the whole-carcass rinsing method than with destructive methods due to "technician fatigue" when broiler carcasses are being shaken manually. In our study, we found that variation between samples was lower for the rinsing method than for the neck-skin method. It should be noted that the number of samples used is relatively low and therefore the conclusion drawn from the present study should be considered cautiously. Nevertheless, as the rinsing method gave the highest recovery of bacteria and the lowest variation between samples in our hands, this method will be used in a future decontamination trial.

When assessing both high recovery, quick and convenient sampling and little equipment needed, neck skin excision was regarded as the most useful method for routine testing and preferred by many abattoirs for monitoring of the process hygiene.

The average contamination levels found on broiler carcasses in this study was a TPC mean of 3.5 log cfu/cm^2 (n = 100), which is similar to values reported in other studies at 3–5 log/cm^2 (Alnajrani et al., 2018; Gill & Badoni, 2005). The *E. coli* level is usually about 1–4 log cfu/cm^2 (Althaus et al., 2017; Gill & Badoni, 2005; Loretz et al., 2010). In this study, the level of *E. coli* was almost as high as Enterobacteriaceae, especially for the rinsing method (4 log cfu/cm^2 compared to 2 for *E. coli* in swabbing). This is probably within normal variation, as large amount of fecal contamination on carcass surfaces produce high numbers of *E. coli* in proportion of Enterobacteriaceae (Althaus et al., 2017; Røssvoll et al., 2017).

The Petrifilm analysis used in this study, is regarded as reliable with high correlation to traditional plating method (Park et al., 2001; Silbernagel & Lindberg, 2002).

Comparison of study results are generally difficult as there are large variations, such as day-to-day variation in slaughter hygiene, despite the slaughter line, operators and line speed being the same, and few standards for sampling methods and analyses are available. Capita et al. (2004, pp. 1303–1308) suggested that correlations between destructive and non-destructive methods should be established for each meat and poultry plant. For broiler carcasses, several studies have attempted to calculate the relationship between excision and rinsing methods (and swabbing), but as far as we know, no conversion factors have yet been established. The results of this study indicated that the recovery by neck-skin sampling (log cfu/cm^2) was about 80–100% of the indicator bacteria compared with using the WCR method. The breast skin and swabbing methods each recovered about 50–65% of the bacteria that were found using the WCR method. This study results confirm the necessity of providing more clearer guidelines for broiler carcass sampling for more uniform approach when quantifying carcass contamination.

5. Conclusion

In this study, four sampling techniques for determining the microbial quality of broiler carcasses were compared. The WCR method of sampling gave the highest recovery of Enterobacteriaceae and *E. coli*, followed by neck skin excision, breast skin excision, and swabbing. Neck skin excision was the quickest method and is useful in routine monitory testing.

CRediT authorship contribution statement

Gunvor Elise Nagel Gravning: Conceptualization, Methodology, Software, Writing - review & editing. **Ole-Johan Røtterud:** Conceptualization, Writing - review & editing. **Solfrid Bjørkøy:** Conceptualization, Writing - review & editing. **Merete Forseth:** Conceptualization. **Eystein Skjerve:** Supervision. **Ann-Katrin Llarena:** Writing - review & editing. **Astrid Lian:** Formal analysis, Writing - review & editing. **Gro S. Johannessen:** Writing - review & editing. **Sigrun J. Hauge:** Conceptualization, Methodology, Software, Writing - review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107589.

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