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Microbial ecology on poultry carcasses along the production line*

*Mikrobielle Ökologie von Geflügelschlachtkörpern entlang der Fertigungslinie**

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** Dedicated to Prof. Dr. Karsten Fehlhaber on the occasion of his 65th birthday*
Herrn Prof. Dr. Karsten Fehlhaber zum 65sten Geburtstag gewidmet

Summary

In this study we analysed the microflora of chicken feces during the fattening period and the flora on the carcasses at the main slaughter processing steps, using traditional cultural microbiological techniques. There was no relation between composition and concentration of contaminant (enteric) bacteria in feces and on eviscerated carcasses. No significant changes of concentrations of *Enterobacteriaceae, E. coli,* Enterococci, Staphylococci, Lactobacilli and *Pseudomonas* were observed after scalding, defeathering and evisceration, whereas during chilling the average counts decreased for ≥ 0.9 log for all bacterial groups except Staphylococci. None of these bacterial groups was found suitable as an indicator for the contamination of the carcass with bacteria originating from feces, feathers or skin.

Pooled fecal samples were taken from flocks in weekly intervals during the entire fattening period and subsequently tested for *Salmonella* and *Campylobacter.* The presence of these pathogens in feces [always resulting in carcasses being contaminated (i. e. ca. 18 % for *Salmonella* and 85 % for *Campylobacter*)], proved to be unsuitable as a useful predictor, as "false negative" rates were 62.5 % for *Salmonella* and 25 % for *Campylobacter.*

It is suggested that the above mentioned limitations can be overcome by novel techniques profiling microbial DNA rather than by classical microbiology determining bacterial numbers at genus or species level. In particular promising are 16S RNA genes analysis for microbial communities or specific RT-PCR for obtaining quantitative data.

Keywords: Chicken slaughter, indicator bacteria, *Salmonella, Campylobacter,* cultural microbiology

Zusammenfassung

In dieser Arbeit wurden die Mikroflora von Hühnerkot während der Mastperiode und die Mikroflora der Schlachttierkörper nach den wesentlichen Prozessstufen der Schlachtung mittels traditioneller kultureller Methoden untersucht. Zwischen der Flora in den Fäces und jener auf dem Schlachttierkörper konnte weder hinsichtlich Keimspektrum noch Konzentration ein Zusammenhang hergestellt werden. Während der Prozeßstufen „Brühen", „Rupfen" und „Ausweiden" kam es zu keinen signifikanten Änderungen der Enterobacteriaceen-, *E. coli-,* Enterococcen-, Staphylococcen-, Lactobacillen- und *Pseudomonas*-Konzentrationen; nach der Kühlung konnte allerdings (mit Ausnahme der Staphylococcen) eine Reduktion ≥ 0,9 $\log_{10}$ nachgewiesen werden. Keine der untersuchten Bakteriengruppen eignete sich in dieser Studie als Indikator für eine von Haut/Federn bzw. den Fäces herrührende mikrobielle Kontamination der Schlachttierkörper.

Von den Herden wurden in wöchentlichen Abständen Sammelkotproben auf *Salmonella* und *Campylobacter* untersucht. Bei den Herden mit positiven Kotproben wurden auch regelmäßig *Salmonella* bzw. *Campylobacter* auf den Schlachttierkörpern isoliert, allerdings mit unterschiedlicher Häufigkeit (ca. 18 % für *Salmonella* und 85 % für *Campylobacter*), der Nachweis im Kot war aber kein zuverlässiger Indikator, da die „Falsch-negativ" Raten 62,5 % für *Salmonella* und 25 % für *Campylobacter* betrugen.

Die geschilderten Einschränkungen kultureller mikrobiologischer Verfahren können mittels neuartiger molekularbiologischer Techniken überwunden werden. Im Speziellen gilt die Untersuchung der 16S RNA Gene für bakterielle Gemeinschaften als wertvoll ebenso wie die Emittlung von quantitativen Daten mittels RT-PCR.

Schlüsselwörter: Hühnerschlachtung, Indikatorbakterien, *Salmonella, Campylobacter,* kulturelle Mikrobiologie

Introduction

Poultry is one of the major meat species worldwide, and its contribution to the total meat consumption is increasing (Speedy, 2003). E. g. in Austria, the annual *per capita* consumption of poultry meat increased from 9.1 kg in 1995 to 12.0 kg in 2005 (Anonymous, 2008) and is now second only to pork, whereas in the same period, beef and veal consumption decreased from 13.0 to 12.0 kg.

As all other meats, poultry meat has been implicated in human foodborne disease, but in the EU, meat from poultry in particular is accounting for ca. 24–29 % of human cases of foodborne Campylobacteriosis (EFSA, 2010) and to a similar extent (estimates ranging from 15 to > 25 %; EFSA, 2008) for Salmonellosis. Poultry may be symptomless carriers – i. e. they may harbour these pathogens in their intestines – and via the release of fecal material during transportation, birds may become contaminated on the outside (ICMSF, 1998). A further spread of enteric bacteria occurs during scalding (Barros et al., 2007) – its extent being dependent on the water temperature and scalding duration (ICMSF, 1998) – as does defeathering, both causing cross contamination of carcasses with microflora on the surface of the carcasses (Allen et al., 2003). Hence, the latter processing step is regarded as a significant contamination site as regards Staphylococci and other Gram positives (ICMSF, 1998), whereas during evisceration, feces and thus, enteric pathogens can be transferred to scalded skin as well as to muscle tissue. Although such contamination routes are well known, further data are necessary to describe the transfer of enteric pathogens at different process steps in the slaughterline in more quantitative terms (Evers, 2004; Nauta et al., 2005). This applies also to bacteria indicating fecal contamination and thus, the potential presence of enteric pathogens (indicator bacteria, as are *E. coli* and Enterococci; Schaffner and Smith, 2004). Also, little is known about the extent to which autochtonous spoilage bacteria originating from the intestines or feathers/skin find their way on the dressed carcass and about the selective pressure exerted by slaughter processing steps on this flora (Notermans et al., 1977).

The purpose of this study was to (1) compare the microflora of chicken feces with that found on eviscerated carcasses; (2) to characterize in quantitative terms the microbial ecology on the carcass surfaces at the main chicken slaughter processing steps, in an attempt to estimate the contribution of bacterial populations on the skin/feather and intestines to the microflora found on the dressed carcass and to assess which processing steps contribute to these contamination events; (3) in addition, the presence of *Campylobacter* and *Salmonella* in naturally contaminated flocks was studied, so as to assess the quantitative relationship of their presence in feces at flock level and their prevalence on the carcasses after slaughter. The work exclusively reports our results obtained with the classical cultural microbiology approach, i. e. discriminating bacteria at genus or species level.

Materials and methods

Samples

Samples originated from a total of 30 Austrian chicken flocks (26 from conventional and four from organic farming). The comparison of fecal and carcass microflora was done with six flocks, monitoring of microbial populations along the slaughterline and relation of *Campylobacter* and *Salmonella* in feces and on carcasses in each twelve flocks. In the first week of fattening, transport diapers were sampled and in the following weeks, up to the day prior to slaughter, pooled fecal samples were taken in weekly intervals. Slaughter was performed at two different operations. Three to ten carcasses were randomly sampled per flock at the end of each of the following processing steps: „scalding“, „defeathering“, „evisceration“, „cooling“, „packaging“ (i. e. placing on styrofoam trays and wrapping in oxygen-permeable foil). Fecal samples as well as carcasses were transported to the laboratory under refrigeration and analysed at the day of arrival.

Microbiological examination

Serial dilutions were prepared in maximum recovery diluent (MRD; Oxoid) from a 10 g aliquot of each pooled fecal sample. Carcasses were placed in sterile plastic bags, then 300 ml sterile saline (0.85 % NaCl) were added and the bags were closed and shaken vigorously for 2 min. From the rinse, serial dilutions were prepared in MRD and 50 ml aliquots were taken for detection of *Campylobacter* sp. and *Salmonella* sp.

Samples were tested for the presence of *Campylobacter* sp. and *Salmonella* sp. and numbers of *E. coli, Enterobacteriaceae,* Enterococci, Staphylococci, *Lactobacillus* sp. and *Pseudomonas* were determined according to the methods described in Table 1.

Statistical analyses

Bacterial concentrations were converted into log cfu per ml (carcass rinse) or per g (feces). Differences in microbial numbers beween slaughter processing steps were assessed using the Kolmogorov-Smirnov and the Kruskal-Wallis test. Differences between slaughterhouses and rearing conditions were assessed using the Kolmogorov-Smirnov and the Mann-Whitney-U test. Calculations were done by SPSS for Windows software, V.14; significance was established at $P< 0.05$.

TABLE 1: *Microbiological methods for the analysis of fecal samples and carcass rinses.*

Parameter	Method
E. coli	Direct plating onto Coli ID agar (BioMerieux), incubation 37 °C, 24h; biochemical confirmation of randomly selected subcultured typical colonies (API 20E, BioMerieux))
Enterobacteriaceae	VRBG agar (BioMerieux) acc. ISO 21528-2 (2004), but only oxidase reaction for colony confirmation
Enterococcus sp.	Chromocult Enterococci agar (Merck), incubation 42 °C, 24 h (Manafi and Sommer, 1993)
Staphylococcus sp.	KRANEP agar (Merck), incubation 37 °C, 48 h (Sinell and Baumgart, 1967)
Lactobacillus sp.	Colony counting on Rogosa agar (Merck), incubation 30 °C, 120 h, anaerobic (Rogosa et al., 1951)
Pseudomonas sp.	Colony counting on GSP agar (Merck), incubation 25 °C, 72 h (Kielwein, 1969)
Campylobacter sp.	ISO/TS 10272 (2006)
Salmonella sp.	Enrichment in buffered peptone water (Oxoid), incubation 37 °C, 24 h; streaking onto XLT and MacConkey agar (Oxoid), incubation 37 °C, 24 h and additional motility testing on MSRV agar (Oxoid), incubation 42 °C, 24 h; serological testing (polyvalent I serum, Dade Behring)

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Results and discussion

Differences between rearing conditions and slaughterhouse operations

No significant differences were found for microbial numbers as regards mode or rearing (organic vs. conventional). Significant differences were found between the two slaughterhouses after the processing steps "scalding", "defeathering" and "evisceration" (data not shown). Differences in the layout of the plant and design of machinery, scalding temperature or frequency of water washes may have accounted for that (ICMSF, 1998).

TABLE 2: *Microbiological profile of chicken feces at the day before slaughter and on eviscerated carcasses and correlation r of results (data from six flocks).*

	Feces (log cfu/g)	Carcass (log cfu/ml rinse)	r
Enterobacteriaceae	6.67 ± 0.72	5.18 ± 0.55	–0.30 (P = 0.57)
E. coli	6.56 ± 0.64	5.66 ± 0.59	0.25 (P = 0.63)
Lactobacilli	7,43 ± 0,54	5.50 ± 0.48	–0.25 (P = 0.55)
Enterococci	6.84 ± 0.75	4.93 ± 0.66	–0.53 (P = 0.29)
Staphylococci	7.50 ± 0.62	4.81 ± 0.27	–0.55 (P = 0.26)

Relation of fecal flora and contaminant flora on eviscerated carcasses

Average bacterial concentrations in feces are reported in Table 2. Correlations (r) to the counts on carcasses were only weak and not statistically significant. Likewise, El-Ghareeb et al. (2009), examining wild bird carcasses which had been eviscerated manually, failed to establish a stringent relation between microbial numbers in feces, on feathers and on dressed carcasses of wild birds.

When studying Table 2 column-wise, it becomes evident that the relation of concentrations of Lactobacilli, Enterococci and Staphylococci to *Enterobacteriaceae* (or *E. coli*) in feces differs from that observed on carcasses, indicating a slight shift toward Gram negative bacteria. This was not expected as the slaughter process might indeed favour Gram positives: scalding would select for Gram positive bacteria generally being more resistant to heat than Gram negatives and Staphylococci and Micrococci be transferred from the rubber fingers of defeathering equipment to the carcass skin (ICMSF, 1998; Allen et al., 2003). Also, the relatively higher numbers of Gram positives in feces as compared to *Enterobacteriaceae* would mean that fecal contamination during evisceration would further increase concentrations of Gram positives on carcasses.

Microbiological profile of chicken carcasses at major steps of the slaughterline

Microbial numbers obtained at the end of the processing steps „scalding", „defeathering", „evisceration", „cooling", „packaging" are displayed in Table 3. The method of sampling was quite comparable to that used in other studies (Russell et al., 1997; Allen et al., 2003; Handley et al., 2010). However, the average bacterial numbers reported by Handley et al. (2010) for dressed carcasses were markedly lower: e. g. 0.66 log cfu/ml for *Enterobacteriaceae* and 0.53 log cfu/ml for *E. coli.* Respective numbers in our study were 4.16 and 4.05 log cfu/ml.

Regarding the processing steps, significantly lower bacterial numbers were found for the steps "refrigeration" and "packaging" as compared to the previous processing steps.

In our study, there was no significant effect of scalding on microbial numbers. This can be explained by the fact that "soft scald", as commonly applied for poultry that is intended to be sold chilled and not frozen (Richmond, 1990) is done at temperatures around 50–55 °C (ICMSF, 1998). In this temperature range, both significant reductions > 1 log cfu (ICMSF, 1998) and constant numbers of bacteria (Notermans et al., 1977) have been reported. Obviously a high number of enteric bacteria surviving scalding will not allow to use these bacteria as markers for fecal contamination occurring at subsequent processing steps (ICMSF, 1998), as was also observed in our study.

Defeathering is known to increase total aerobic counts and numbers of *E. coli* as well as the prevalence of certain pathogens on the carcass skin (reviewed by ICMSF, 1998). In our study, such increases were not observed for the six groups of bacteria studied with quantitative methods.

Air chilling has been demonstrated to reduce surface counts for various meat species (for pig: see Snijders et al., 1984; poultry: Lillard, 1990; beef: Gill and Bryant, 1997; mutton: Loncaric et al., 2009). The rationale is that lowering of temperature will retard bacterial growth and reducing a_w on the surface will reduce bacterial viability. However, only few reports indicate that cooling of chicken carcasses with air would reduce bacterial numbers for more than 0.5 log (e. g. James et al., 2006). In our study, average reductions were ≥ 0.9 log cfu/ml, with exception of Staphylococci, with a lower reduction of ca. 0.5 log (Tab. 3).

In sum, the numbers of "indicator bacteria" remained roughly the same at the steps "scalding", "defeathering" and "evisceration". Thus, they gave no indication if contamination / cross-contamination of carcasses had occurred and also had no predictive value for the presence of *Campylobacter* or *Salmonella.* By the same token, it should be realized that the concept of autochthonous indicator bacteria – rather than being useful for indicating the presence of pathogens in raw meats (e. g. Loncaric et al., 2009) – principally serves to identify if ultimately recontamination of processed products has taken place (Schaffner and Smith, 2004).

Campylobacter and *Salmonella* in chicken feces before slaughter and on carcasses

The early detection of enteric pathogens at flock level prior to slaughter could allow to slaughter flocks under special

TABLE 3: *Microbiological profile of chicken carcasses at major steps of the slaughterline (log cfu/ml rinse; mean±std.dev.; data from twelve flocks).*

	Scalding	Defeathering	Evisceration	Refrigeration*	Packaging*
Enterobacteriaceae	5.86 ± 0.71	5.27 ± 0.40	5.12 ± 0.52	4.01 ± 0.41	4.16 ± 0.21
E. coli	5.91 ± 0.83	5.16 ± 0.45	5.63 ± 0.62	3.83 ± 0.41	4.05 ± 0.35
Pseudomonas	6.08 ± 0.58	5.71 ± 0.62	5.57 ± 0.56	4.64 ± 0.34	4.85 ± 0.16
Lactobacilli	6.16 ± 0.36	5.43 ± 0.36	5.48 ± 0.36	4.58 ± 0.30	4.68 ± 0.27
Enterococci	5.95 ± 0.63	5.15 ± 0.52	4.91 ± 0.61	3.92 ± 0.39	3.75 ± 0.52
Staphylococci	6.59 ± 0.75	5.37 ± 0.63	4.79 ± 0.31	4.20 ± 0.38	4.29 ± 0.15

*Results from processing steps with asterisk differ significantly from those without asterisks

sanitary conditions in order to avoid cross-contamination of following flocks slaughtered on the same line. Such concepts (logistic slaughter) require (1) that samples represent the flock status near to slaughter, but also (2) that analytical results are provided at a time where the order of slaughter can be managed and finally, that the miss rate (i. e. false negative results) is as low as possible. The latter issue is of course related to the sensitivity of sampling as well as of detection methodology, which becomes evident when we consider that, in infected flocks, *Campylobacter* concentrations in feces are > 6 log cfu/g, whereas they are substantially lower for *Salmonella* (data not shown).

However, even for *Campylobacter,* a miss rate of 25 % as reported in our study (Tab. 4) leaves something to be desired, especially as the presence of *Campylobacter* in feces at flock level will result in nearly all carcasses (~85 %) being contaminated with this pathogen (as compared to an average of 18 % for *Salmonella*).

TABLE 4: *Presence of Campylobacter and Salmonella in chicken feces before slaughter and on carcasses (data from twelve flocks).*

Flock	*Campylobacter**		*Salmonella***	
	Feces***	Carcasses: n pos/ n analyzed	Feces***	Carcasses: n pos/ n analyzed
1	+	49/49	–	15/49
2	–	0/34	–	0/34
3	+	28/28	–	0/28
4	+	24/24	–	2/24
5	+	38/38	–	0/38
6	–	9/21	–	2/21
7	–	0/15	–	1/15
8	–	0/36	+	6/36
9	–	18/36	+	4/36
10	+	28/33	+	8/33
11	+	28/33	–	6/33
12	–	0/18	–	0/18

*agreement 83 %; false negative rate 25 %; **agreement 58 %; false negative rate 62.5 %; ***positive (+) means that the respective pathogen was detected in at least one of the pooled samples taken during the fattening period

Another option would be a classification of flocks and farms (on the results of repeated flock examination) as done in the Danish pork *Salmonella* control progam, followed by a scheduled slaughter.

In essence, our data confirm that – although *Campylobacter* is frequently isolated at flock level and that this will result in most of slaughter carcasses being contaminated (albeit in sometimes low concentrations per carcass) – testing of pooled feces samples is not always sensitive enough to identify all positive flocks. Another drawback is, that testing for *Campylobacter* with classical cultural methods requires ≥ 72 hrs. This dictates that flocks must be tested > 3 days before slaughter and hence it cannot be excluded that the flock's status has changed during these three final days prior to slaughter.

Conclusion

Possibilities and limitations of cultural microbiology

The present study demonstrates the limitations of traditional microbiological techniques when microbial communities exposed to a sequence of processing steps are to be characterized. This relates to the fate of the autochthonous flora of the poultry as well as to the flora presumably residing on equipment, utensils and the workers. Several approaches have been used to overcome this problem, for example the artificial introduction of defined bacteria in the slaughterline (e. g. *E. coli* K12; Mead et al., 1994; Allen et al., 2003) or the monitoring of particular bacterial genera or species (e. g. Bifidobacteria, Delcenserie et al., 2008; Loncaric et al., 2009). However, the latter usually requires molecular biological methods to allow for clearly identifying and tracing bacteria. While these approaches may be useful to identify the sites and extent of contamination, they inherently fail to reflect the changes in microbial communities, which one would expect as a result of effects of temperature (scalding, chilling), pH (scalding) and reduction of a_w (chilling). Generally, cultural microbiological techniques will reveal only a small part of the spectrum of the intestinal flora (Suau et al., 1999), and it has been suggested that, alternatively, analyses of DNA, in particular 16s rRNA, would be far more effective to remedy this flaw (Schwieger and Tebbe, 1998). This concept has already been applied in poultry to monitor the changes occurring during chilling and temperature abuse of dressed carcasses (Handley et al., 2010). Yet, there is, to our knowledge, no study exploring the potential of such methodology to characterize microbial communities along the poulty processing chain. Results of a parallel study using the latter approach will be reported separately (Hilbert et al.; in preparation).

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