POTENTIAL SOURCES OF *CHRYSEOBACTERIUM* CONTAMINATION DURING POULTRY PROCESSING: A PILOT STUDY

H. DE BEER AND C.J. HUGO

ABSTRACT

The genus *Chryseobacterium* is often found in food and is regarded as a food spoilage organism. In this study, the source of the chryseobacteria was uncertain. As an exploratory investigation, the potential source of chryseobacterial contamination was determined. Total bacteria counts and yellow-pigmented colony counts were performed. *Chryseobacterium* species were present on poultry carcasses at all stages of processing. Total *Chryseobacterium* counts increased from 5.6 to 11.8 % after the brine injection stage. A significant increase in total *Chryseobacterium* counts (20.0 and 25.2 %) in the processing waters occurred where cutting up of the carcasses was involved. It is speculated that live chickens are the source of contamination.

Keywords: *Chryseobacterium*, food contamination, processing.

1. INTRODUCTION

Several studies revealed that apart from the Gram-positive strains, several Gram-negative strains are present on poultry, including *Pseudomonas* spp., *Flavobacterium* spp., *Alcaligenes* spp., *Klebsiella* spp. and *E. coli* (Geornaras *et al.*, 1996; García-López *et al.*, 1998, 1999; Hang'ombe *et al.*, 1999; Hinton *et al.*, 2004; Vazgezer *et al.*, 2004). *Pseudomonas* and flavobacteria are Gram-negative, aerobic rods capable of growing under refrigerated conditions. Conditions and incidences of *Pseudomonas* and *Flavobacterium* on chicken carcasses were 17% and 16% respectively (Mai and Conner, 2001). Nychas and Drosinos (1999) found that the incidence of flavobacteria on poultry is much higher compared to that of other fresh meat. Metabolites produced by these bacteria include alcohols, sulphur compounds, ketones, aldehydes, esters and amines and the resultant odours can be described as fishy, foul, sulphuric and ammonia like (Nychas and Drosinos, 1999; Mielmann, 2006). According to Mead (1989), micro-organisms enter the poultry processing plant through feathers, skins and intestines which accumulate in the processing water. Several processing steps reduce the contamination levels but equipment and operating procedures may contribute to the microbiological load of the poultry end product (Geornaras *et al.*, 1996). A significant increase of psychrotrophic spoilage bacteria during processing is due to cross-contamination from the air, water, handling practices and intestines. These bacteria are responsible for spoilage of poultry during refrigerated storage (Hinton *et al.*, 2004). Competition between the different micro-organisms on the food, as well as the temperature of storage, will also

have an effect on bacterial populations and food spoilage (Forsythe, 2000; Jay, 2000).

Originally *Flavobacterium* consisted of 46 yellow-pigmented, mainly Gram-negative, species (Bergey *et al.*, 1923). The taxonomy of the flavobacteria has undergone many changes especially since 1994 and many of the former *Flavobacterium* species now belong to the genus Chryseobacterium which is only one of several genera in the *Flavobacteriaceae* family (Bernardet *et al.,* 1996; 2002). Significant genera in terms of food microbiology in this family include *Bergeyella*, *Chryseobacterium*, *Empedobacter, Myroides* and *Weeksella* (Hugo and Jooste, 2003). The genus *Chryseobacterium* is widely distributed in the environment and is often associated with food spoilage (Bernardet *et al.*, 1996). Due to this fairly new reclassification, literature regarding food spoilage still refers to *Flavobacterium*/flavobacteria as psychrotrophic bacteria responsible for spoilage. Another concerning factor is the potential pathogenic feature of some *Chryseobacterium* species especially *C. gleum* and *C. indologenes* (Pavlov *et al.*, 2004; Bernadet *et al.*, 2005; Cascio et al., 2005; Michel et al., 2005; Bayraktar et al., 2007; van Wyk, 2008). *Chryseobacterium indologenes* was implicated in conjunctivitis in Switzerland and it is believed that water systems in the hospital were responsible for contamination (Lu and Chan, 1997). Burn wound infections and fatalities in England in 2001 were caused by unidentified *Chryseobacterium* species and in two cases the source of contamination was untreated water (Fraser and Jorgensen, 1997; Kienzle *et al.*, 2001).

During a previous study, the taxonomic heterogeneity of flavobacterial strains in poultry, red meat and fish was determined (De Beer, 2005). It was clear that several *Chryseobacterium* species within the flavobacteria group were present on raw chicken samples (De Beer *et al.*, 2005). In order to obtain a better indication of the origin of the chryseobacteria, a pilot study was done to determine the incidence of flavobacteria during the different processing activities in a broiler-processing factory.

2. MATERIALS AND METHODS

2.1. Sampling points

Sampling was done in a high throughput poultry abattoir (Bloemfontein, South Africa) with a slaughtering capacity of 65 000 to 67 000 birds per day. Birds came from contracted poultry farmers or the company's own farm. Microbiological contamination of the bird carcasses and water used during processing was investigated. All samples were chosen at random from the production line at 5 min. intervals between the different sampling points.

2.2. Sample collection

Surface swab samples on whole bird carcasses were taken at two different

stages in the production line, namely after the first washing step but before evisceration and after the brine injection stage. The swabs taken at these two stages were from each carcass's neck, back, right wing and right thigh (1 cm^2). After the brine injection stage, three samples from the internal part of the carcass were also taken. Swabs were immersed in 9 ml sterile quarter strength Ringer solution (Oxoid BR52) and kept at low temperature (<7 °C) until further investigation. Microbial analyses were done within 2 but not longer than 5 hours from sampling. Table 1 contains a summary of the sampling plan.

Processing water samples were taken at different stages of the production process in sterile containers and as indicated in Table 1. Two samples of the processing water were taken at each step: before contact with the chicken carcass and the water dripping from the carcass. The samples were kept at low temperature until further investigation. Microbial analyses were done within 2 but not longer than 5 hours from sampling.

Table 1: Sample plan

Sampling point	Number of samples	Comments
Surface swab (1cm^2)	**n = 41**	
Whole bird sampled – head, feathers, feet removed, after pre-washing	20	Five birds, external swabs (four areas on carcass)
Whole bird sampled – intestines removed , after brine injection	21	Three birds, external and internal (four areas on carcass, three inside)
Water sample	**n = 36**	
Pre-washer	7	Three before contact with carcass; four during process (water dripping from carcass)
After evisceration	7	Two before contact with carcass; five during evisceration (water dripping from carcass)
End of spin chiller	6	Two at start of process; four at end of spin chilling process
Cutting of whole carcasses	8	Four before contact with carcass; four during cutting process (water dripping from carcass)
Cutting into portions	8	Four before contact; four after portions cut (water dripping from portion)

2.3. Microbial analysis

2.3.1. Total bacteria and yellow-pigmented colony counts

Serial dilutions of the surface swab samples and the water samples were prepared in 9 ml quarter strength Ringers' solution and spread-plated onto plate count agar (in duplicate). All the plates were first incubated at 4°C for 24 hours and then at 25°C for 48 h. Total aerobic plate counts, as well as total yellow colony counts were recorded from plates with counts of between 30 and 300 colonies. The total yellow colony count represents the flavobacteria group. Reference strains representing different species within the *Flavobacteriaceae* family were acquired from the culture collection of the University of Ghent (LMG Bacteria Collection, Laboratory for Microbiology, University of Ghent, Belgium).

2.3.2. Screening for *Chryseobacterium* species

All yellow and orange-yellow colonies from the total bacteria count plates were inoculated on brilliant green agar (Oxoid CM263) and incubated at 25°C for 48 hours. Yellow colonies turning the surrounding medium red were regarded as potential *Chryseobacterium* species. Yellow colonies were regarded as *Chryseobacterium* strains when exhibiting shiny, orange to yellow colonies with entire edges and when the cells were Gram-negative, rod-shaped, non-motile, produced catalase, oxidase and a flexirubin-type pigment using the KOH method (Bernardet *et al.*, 2002; Hugo and Jooste, 2003).

2.4. Statistical analysis

Statistical analyses were done on the log counts of the total bacteria counts, total yellow-pigmented colony counts and the total *Chryseobacterium* counts with one-way analysis of variance using Duncan's multi-stage test, for a confidence interval of 95% using STATISTICA (data analysis software system), version 7.1 (StatSoft, Inc., 2006). Significant differences are indicated with a superscript symbol. Results of actual counts (Table 2) are expressed as means standard deviation and statistical analyses were calculated with log transformed values.

Table 2: Summary of microbial counts on chicken carcasses and in process water obtained during chicken processing

	n	Total bacterial counts (mean ± standard deviation) (log cfu/ml)			Average Percentage (%) of Total Bacterial Count	
		Plate count	Yellow colonies	*Chryseobacterium* colonies	Yellow colonies	*Chryseo-bacterium*
Surface swabs from birds	**41**					
After scalding: surface	20	$9.25 \times 10^3 \pm 7.8 \times 10^3$	$1.02 \times 10^3 \pm 9.4 \times 10^2$	$5.15 \times 10^2 \pm 4.9 \times 10^2$	11.0	5.6
After brine injection: surface	12	$3.54 \times 10^3 \pm 3.1 \times 10^3$	$5.88 \times 10^2 \pm 7.8 \times 10^2$	$4.17 \times 10^2 \pm 6.6 \times 10^2$	17.0	11.8
After brine: intestines	9	$9.11 \times 10^3 \pm 5.2 \times 10^3$	$5.72 \times 10^2 \pm 8.7 \times 10^2$	$4.45 \times 10^2 \pm 7.2 \times 10^2$	6.0	4.9
After brine: combined	21	$5.93 \times 10^3 \pm 3.1 \times 10^3$	$5.81 \times 10^2 \pm 7.8 \times 10^2$	$4.29 \times 10^2 \pm 6.6 \times 10^2$	9.8	4.9
Process water	**36**					
Before processing	15	$1.00 \times 10^1 \pm 0.0$	$0.0 \times 10^0 \pm 0.0$	$0.0 \times 10^0 \pm 0.0$	1.0	0.0
After evisceration	5	$4.00 \times 10^4 \pm 2.9 \times 10^{4\,\#}$	$2.00 \times 10^3 \pm 1.9 \times 10^{3\,\#*}$	$8.00 \times 10^2 \pm 1.3 \times 10^{3\,*}$	4.0	1.9
Pre-washer start	4	$3.00 \times 10^5 \pm 5.44 \times 10^{4\,\#}$	$9.00 \times 10^3 \pm 8.3 \times 10^{3\,\#}$	$4.00 \times 10^3 \pm 5.0 \times 10^3$	3.0	1.7
Spin chiller end	4	$8.00 \times 10^1 \pm 0.0$	$0.0 \times 10^0 \pm 0.0$	$0.0 \times 10^0 \pm 0.0$	1.0	0.0
After cutting	4	$2.30 \times 10^3 \pm 3.7 \times 10^3$	$5.00 \times 10^2 \pm 1.0 \times 10^3$	$5.00 \times 10^2 \pm 1.0 \times 10^3$	20.0	20.0
After portions	4	$4.00 \times 10^4 \pm 2.5 \times 10^{3\,\#}$	$1.00 \times 10^4 \pm 1.0 \times 10^{4\,\#*}$	$1.00 \times 10^4 \pm 9.1 \times 10^{3\,*}$	32.0	25.2

$^{\#}$ Superscripts indicate significant differences between all the groups for Total counts and Total Yellow counts ($P \leq 0.05$)

* Superscripts indicate significant differences between all the groups for Total Yellow counts and Total *Chryseobacterium* counts ($P \leq 0.05$)

3. RESULTS AND DISCUSSION

The results of the total bacteria, yellow-pigmented colonies and *Chryseobacterium* counts from surface swabs of the carcasses and from processing water obtained from the different stages of processing are presented in Table 2. Fresh potable water was introduced at several processing areas throughout the plant. As water is frequently replaced with new potable water, contamination levels fluctuated in different processing areas. To exclude the water as source of contamination, counts were done at the beginning and the end of each processing step. All the water samples done before carcass contact had counts of less than 10 cfu/ml. It was therefore assumed that the processing water as such was not the major source of contamination. The before-processing water was therefore not included in the statistical analysis. Cold temperatures at the spin chiller process could explain the lower reported counts. During poultry processing the load of total bacteria in the processing water dripping from the carcasses increased significantly ($p < 0.05$), specifically during processes where cutting of the carcasses was involved. At the two areas a significant increase in the total yellow colony count and total *Chryseobacterium* count occurred ($p < 0.05$). At both of these processes the total yellow colonies and total *Chryseobacterium* count respectively represent 20.0, 32.0, 20.0 and 25.2% of the total bacteria count (Table 2). Mead (1989) and Geornaras *et al.* (1996) reported on the role of cross-contamination by psychrotrophic bacteria during processing from equipment, processing water and handling. They confirmed the occurrence of *Flavobacterium* and *Cytophaga* species during poultry processing. As it became evident that many of the *Flavobacterium* species were assigned to the *Chryseobacterium* genus after 1996 (Bernardet *et al.*, 1996; 2002), it could be assumed that many of the yellow colonies might be *Chryseobacterium* species.

Possible explanations for large standard deviations include different levels of initial contamination and the location of swabs on the carcasses, especially underneath the wings, as well as continuous replacement of the processing water with fresh potable water. These problems should be eliminated by a larger sample size.

4. CONCLUSION

During this study it was evident that *Chryseobacterium* species were present throughout the poultry processing procedure. After the brine injection process there was a significant increase in the total yellow colony count (17%) and a subsequent increase in *Chryseobacterium* count (11.8%). *Chryseobacterium* species were present on whole birds throughout the different stages of processing. A significant increase in the total *Chryseobacterium* counts (20.0 and 25.2%) in processing waters occurred during processes where cutting of the carcasses was involved. It is therefore speculated that the original source

of *Chryseobacterium* in a poultry processing plant is the live incoming birds. This should be investigated in future.

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