Spoilage microflora succession on lamb carcasses at chill temperatures

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SUMMARY: The microbiology of lamb carcasses was investigated in order to identify the microbial succession during aerobic refrigeration spoilage as well as the influence of several factors (sampling method, sampling area and sampling day) on their recovery. Three main groups were detected during shelf-life: coryneforms, Gram-positive cocci and Gram-negative bacilli. The sampling method and the sampling day influenced the type of microorganism recovered.

INTRODUCTION: Meat is an excellent substrate for microbial growth, and this fact is a prime factor for its spoilage (GILL, 1986). It has also been proved that under normal conditions this is a phenomenon that takes place on the surface and microbial penetration does not occur until proteolytic enzyme production at the end of the logarithmic phase of growth (GILL and PENNEY, 1977). The usual practice of carcass chilling after dressing accounts for the fact that meat spoilage is mainly associated with microorganisms capable of growing at refrigeration temperatures. Although this flora varies, it is generally recognized that the main spoilage microorganisms are Gram-negative bacilli, specially *Pseudomonas*, *Moraxella*, *Acinetobacter* and several genera of *Enterobacteriaceae* (GILL, 1982; DAINTY *et al.*, 1983), and on certain occasions Gram-positive species such as *Brochothrix thermosphacta* (BARLOW and KITCHELL, 1966).

MATERIALS AND METHODS: A total of 1,200 strains were obtained using random procedures during storage life at 3±1°C and relative humidity of 95±5%, of eight lamb carcasses. Half of them were isolated from plates incubated at 30°C and the other 600 from plates at 7°C. Samples were taken at 0, 5, 10 and 15 days postslaughter and when carcasses were no longer in an acceptable condition (presence of slime or/and off-odours). Three sites (leg, neck and brisket; 15 cm^2 each one) were sampled using three different techniques (swabbing, rinsing and excision), according to KITCHELL *et al.* (1973). Identifications at genus and species level of strains were carried out employing numerical characterization procedures (PRIETO, 1990). The chi-square method was used to determine which factors significantly contributed to the genera of bacteria isolated.

RESULTS AND DISCUSSION: Table I. Numbers and percentages of groups and genera of bacteria isolated in the study.

Groups	No	%	Genera	No	%
Gram + cocci	348	33.3	*Staphylococcus*	270	22.2
			Micrococcus	12	1.0
			Others	66	5.5
Coryneforms	353	29.4	*Brochothrix*	291	24.25
			Curtobacterium	18	1.5
			Aureobacterium	11	0.92
			Others	33	2.75
Gram - bacilli	417	34.7	*Pseudomonas*	282	23.5
			Moraxella	103	8.58
			Psychrobacter	15	1.25
			Others	17	1.42
Other Gram + bacilli NI[a]	25	2.1			
Gram+ coccobacilli NI	14	1.17			
Yeasts	11	0.92			
Others NI	32	2.7			

[a] NI, not identified

Table II. Data obtained (significance of four factors in recovery of genera from plates at different temperatures) in the chi-squared test.

Factors	D.F.[a]	30°C	D.F.	7°C
Carcass	70	$p<0.001$	49	$p<0.001$
Sampling day	40	$p<0.001$	28	$p<0.001$
Sampling area	20	0.5774	14	$p<0.05$
Sampling method	20	$p<0.001$	14	$p<0.001$

[a] D. F., Degrees of Freedom

Data and mathematical results obtained (Table II) indicated that microbial genera detected during chill storage of lamb carcasses depended ($p<0.001$) upon carcass and day of sampling as well as on sampling method and to a lesser extent ($p<0.05$, in psychrotrophs) on sampling area. With regard to the first factor, it is clear that, even taking into account the large influence of storage conditions, the evolution of microbial associations is affected by carcass flora at the moment of slaughter as well as by ratio of the diverse microbial groups. These considerations would explain the important association found between carcasses and genera present on them.

The relationship between the storage period and the isolated strains could also be established, since environmental conditions (temperature, desiccation, pH, nutrients, etc.) are among the factors that influence the dominant flora at one given moment (NOTTINGHAM, 1982; DAINTY *et al.*, 1983). It is clear that chilling will pick up part of the population and desiccation will again select. Nutritive requirements (mainly glucose availability), pH values and competition for substrata and 0_2 will influence microflora succession.

Table III displays the evolution of the more important bacterial genera. In the early stages Gram-negative bacilli dominated, then the coryneform group, and finally, Gram-positive cocci. Their distribution on the basis of recovery temperature shows the preponderance of Gram-positive cocci among mesophiles, while in psychrotrophs Gram-negative bacilli and coryneforms outnumbered alternately, the differences being more obvious at the beginning and at the end.

Table III. Evolution, during shelf life of lamb carcasses, of the principal genera isolated (percentages).

Day	0	5	10	15	spoiled
Brochothrix	10.0	33.3	32.5	26.2	23.8
Pseudomonas	26.2	20.8	25.4	30.8	14.2
Moraxella	22.9	10.0	3.7	4.6	1.7
Staphylococcus	17.1	11.7	19.6	24.6	39.9

Either way, the predominant genera found were dependent on incubation temperature of plates. *Staphylococcus* was mainly present in plates incubated at 30°C; whereas those at 7°C principally contained *Moraxella* and *Pseudomonas*.

If we look at genera distribution (Table III), *Pseudomonas* and *Moraxella* constituted the most common genera detected on fresh dressed carcasses (cold water used for washing, and contaminated wool -from soil- appeared to be the major sources of psychrotrophic bacteria), but afterwards their evolution varied. The first one remained stable, whereas the second slowed down to a steady pace. In intermediate phases, *Brochothrix* led and eventually *Staphylococcus*. With the exception of the first five days of storage, *Staphylococcus* dominated at 30°C. The participation of *Brochothrix*, alternatively with *Pseudomonas*, was also very important.

In our case, the key factor in the evolution of preponderant genera (*Pseudomonas-Brochothrix-Staphylococcus*) could be desiccation because *B. thermosphacta* is more resistant to low a_w than *Pseudomonas*, and *S. xylosus* (the principal species among staphylococci) known for its ability to overcome disgenesic environments (KLOOS *et al.*, 1976a; NOTTINGHAM, 1982). The prevalence of staphylococci (coagulase-negative, novobiocin-resistant) and the absence of certain species susceptible to this antibiotic endorse the observations of KLOOS *et al.* (1976b) who express the adequacy of this group for multiplying in less propitious conditions than man-associated species

Another distinct point is the potential spoilage capability. It seems as if it would be favoured by primary actions due to other more significant microorganisms in the earlier stages.

The efficiency of the sampling methods to assess carcass contamination has often been measured, but its influence on microbial types has been less considered. However, it is reasonable to hypothesize that the bacteria-meat relationship will affect the type and number of microorganisms isolated using a determined method. In our case, the association between sampling methods and genera has been proved mathematically. Rinsing recovered higher percentages of *Moraxella* and *Pseudomonas*, while swabbing was more efficient for *Brochothrix*. The recovery of *Staphylococcus* was not clearly affected either by sampling method or by sampling area. Table IV points out the influence in flora composition depending on sampling areas, though, mathematically, this factor is the least affecting one. Thus, *Brochothrix* numbers were outstanding on the breast, and *Moraxella* and *Pseudomonas* were mainly located on the neck. It is undoubtable that certain factors such as fat tissue distribution or primary contamination due to environmental flora and slaughtering procedures affect and predispose to the establishment of diverse "environmental situations". Nevertheless, desiccation could also play a primary role. Breast trend was to desiccate sooner, and neck retained humidity longer. This distribution has to be born in mind when estimating or evaluating the presence of pathogens on lamb carcasses.

Table IV. Percentages of recovery of different genera in three sampling points and with three sampling methods.

	Neck	Brisket	Leg	Swab	Rinsing	Excision
Brochothrix	22.4	33.7	26.1	24.6	15.3	33.8
Pseudomonas	33.8	23.7	29.6	33.3	39.1	22.1
Moraxella	14.0	8.8	8.7	10.1	16.3	8.3
Staphylococcus	25.0	27.6	26.9	23.7	21.8	30.1
Others	4.8	7.0	8.7	8.3	7.5	5.7

The capacity for non-destructive sampling methods to free different bacterial genera from carcass surfaces was highly variable. Table IV shows that rinsing recovered higher percentages of Gram-negative bacteria. Several researchers (NOTERMANS and KAMPELMACHER, 1975; FIRSTENBERG-EDEN *et al.*, 1978; BUTLER *et al.*, 1979) point out that adhesion of motile Gram-negative bacteria to skin is stronger than that performed by non-motile or Gram-positive ones. This consideration could account for the high percentages of *Pseudomonas* achieved by rinsing and the low figures obtained by *Staphylococcus* and *Brochothrix*, which depended upon the sampling day (higher recovery with swabbing at the final stages, when microcolonies are constituted).

CONCLUSIONS:

- Although the initial composition of each carcass flora influenced microbial succession during chilling, the most common evolution was: first, Gram-negative bacteria, then coryneforms and, eventually, staphylococci.
- Sampling method turned out to be an important factor in bacteria recovery.
- Sampling area less notably affected to genera recovery, this influence due perhaps to the desiccation factor.

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