

# MICROBIOLOGICAL CHARACTERISTICS OF PIG CARCASSES IN ITALIAN SLAUGHTERHOUSES

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**SUMMARY** - The bacterial contamination of pig carcasses and the incidence of *Enterobacteriaceae*, coliforms, *Escherichia coli*, *Salmonella* and group D streptococci were investigated at three slaughterhouses in Northern of Italy. The study included a total of 90 carcasses; four sites on each carcass were studied, two on the shanks and two on the shoulders. There were no differences between mean bacterial loads of all locations examined. The average microbial counts at the three slaughterhouses were  $4.3 \pm 0.6$  log CFU/g,  $4.6 \pm 0.5$  log CFU/g and  $5.0 \pm 0.5$  log CFU/g, respectively.

The *Enterobacteriaceae* counts, regarded as indicators of hygiene during slaughtering, varied considerably among the different samples, ranging from 3 CFU/g to  $1.8 \times 10^5$  CFU/g.

*E. coli*, *Enterobacter agglomerans*, *Serratia marcescens* and *Citrobacter freundii* were predominant among *Enterobacteriaceae*. *Salmonella* spp were found only in four carcasses at one slaughterhouse.

**INTRODUCTION** - The microbial characteristics of fresh pork depends greatly on hygiene during slaughter and sectioning of the carcasses. Evaluation of hygiene has been based largely on the surface bacterial count from the carcasses. Previous research (CHRISTENSEN & SORENSEN 1990) has shown that microbial counts depend on where the sample is taken from, sampling technique and vary from carcass to carcass. The initial contamination of the meat comes mainly from the animal's skin but also from faecal material and from the surrounding environment. The bacterial levels reached during storage depend on the initial contamination as well as the effect of environmental factors.

The aim of this study is to determine the bacterial contamination of pig carcasses and the incidence of *Enterobacteriaceae*, coliforms, *E. coli*, *Salmonella* and group D Streptococci at three slaughterhouses in the North of Italy.

## MATERIALS AND METHODS

**Sampling procedures** - Three slaughterhouses were visited on three occasions and during each visit samples were taken from ten carcasses, after chilling, at four selected points, two on the shanks and two on the shoulders.

At all three slaughterhouses the equipment was cleaned and disinfected before and during our investigation to avoid cross-contamination.

Sampling was done using excision technique.

### Microbiological examination

Surface samples, 4 - 5 mm in thickness and about 20 g in total weight, were homogenized with 0.1% solution of peptone broth buffered at the rate of 1: 3. A series of decimal dilutions of the samples were plated into or spread on various specific media for each microorganism and incubated at the optimum temperature for growth and subsequent counting (Table 1).

Table 1- Type of count, medium used, time and temperature of incubation

MEDIA	T(°C)	HOURS	MICROORGANISMS
Tryptone Soy Agar (Biogenetics) (TSA)	30	72	total aerobic flora
Violet Red Bile Glucose Agar (Biogenetics) (VRBGA)	30	24	Enterobacteriaceae
MUG Violet Red Bile Agar (Biogenetics) (VR-MUG)	37	24	Coliform and E. coli
Kanamycin Aesculin Azide Agar (Oxoid) (KAA)	37	24	Group D Streptococci

For the detection of *Salmonella* 25 g of pork, an average sample from the four sites of each carcass, were transferred into

a flask containing 225 ml of buffered peptone water. After incubation at 37°C for 24 hours, one ml of this culture was inoculated into Salmonella Rapid Test (UNIPATH).

The Enterobacteriaceae were identified using Enterotube II (ROCHE) and API 20E (BIOMERIEUX) following the manufacturers instructions.

*Statistical analysis* - The counts were analysed after transformation into logarithms and submitted to statistical evaluation to obtain an analysis of variance.

## RESULTS AND DISCUSSION

### *Aerobic flora (TSA)*

The distribution of total microbial count in the three slaughterhouses is reported in Table 2.

Total counts ranged from  $5.6 \times 10^2$  CFU/g to  $1.7 \times 10^6$  CFU/g and were mainly due to Gram-negative bacteria. This result agreed with that of NORTJEY et al (1990) that Pseudomonas spp are dominant in the microbial population associated with carcasses. With reference to the distribution 96.7% of the samples at slaughterhouse 1, 93.3% at No. 2 and 85.8% at No. 3 showed values lower than or equal to 5.5 Log CFU/g.

Only 1 sample (0.8%) at the slaughterhouse 1, 2 (1.6%) at No. 2 and 2 (1.6%) at No. 3 exceeded  $10^6$  CFU/g.

Significant differences were between the three slaughterhouses with a F-Test ratio of 39.58 (Table 3).

There was no difference between mean bacterial loads either from samples of shanks or from samples of shoulders; this was in agreement with the work of SNIJDERS et al (1984) where, by the excision technique, no significant differences were observed between each of the four sites on a carcass.

The averages of total counts appeared higher than data of GARDNER (1982) and CHRISTENSEN & SORESENSEN (1991) but the difference of sampling techniques is important to note. Counts per square centimeter are usually based on surface sampling technique such as swabbing that removes only part of total flora (SNIJDERS et al 1984).

Table 2 - Distribution of total counts at the three slaughterhouses

slaughterhouse		1		2		3	
		freq.	%	freq.	%	freq.	%
range							
from to below							
(Log CFU/g)							
2.5	3.5	14	11.6	3	2.5	1	0.8
3.5	4.5	52	43.4	52	43.3	19	15.8
4.5	5.5	50	41.7	57	47.5	83	69.2
5.5	6.5	4	3.3	8	6.7	17	14.2

freq. = n° samples

% = % samples

### *Enterobacteriaceae (VRBGA)*

Two samples at slaughterhouse 1, three at No. 2 and ten at No. 3 showed a count of Enterobacteriaceae of  $< 3$  CFU/g that is our limit of detection. The Enterobacteriaceae counts, regarded as indicators of hygiene during slaughter, varied considerably among the other different samples ranging from 3 CFU/g to  $1.8 \times 10^5$  CFU/g (table 4). 61.5% of samples at slaughterhouse 1, 65.2% at No. 2 and 78.1% at No. 3 had Enterobacteriaceae counts of  $< 10^3$  CFU/g.

Even though the Enterobacteriaceae averages, relative to the total count, were low (table 3) there were some samples with high concentration. It is difficult to define the number of Enterobacteriaceae for an acceptable bacteriological condition of carcasses. GERATS (1987) derived a limit of  $< 1.3$  Log CFU/cm<sup>2</sup> for at least 40% of carcasses.

Table 3 - Microbial count (MTC) and Enterobacteriaceae (Ent.) from the samples taken at the three slaughterhouses, average (Av.) and standard deviation (St. d.)

SLAUGHTERHOUSES	1		2		3	
	MTC	ENT	MTC	ENT	MTC	ENT
Av	4.34	2.74	4.60	2.54	5.01	2.33
St. d.	0.64	0.83	0.62	0.98	0.48	0.97
min.(Log CFU/g)	2.75	1.08	3.36	0.48	3.32	0.48
max.(Log CFU/g)	6.23	5	6.04	4.48	6.11	5.26

#### Salmonella

Although pork is considered one of the sources of *Salmonella* spp, only four carcasses, at the slaughterhouse 1, out of 90 analysed at the three slaughterhouses (4.4%) were found positive for salmonellae.

Data on the presence of *Salmonella* in healthy swine vary considerably; MACKINLEY et al (1980) found salmonellae in faecal samples from 16 of 27 swine farms, SWAMINATHAN et al (1978) found the incidence in pork products to vary between 13 and 19%, BARBUTI et al (1991) detected salmonellae in 15% of fresh meat samples.

#### Coliforms, *Escherichia coli* (VR-MUG) and *Enterococcus* (KAA)

The distribution of the found values are presented in Fig. 1; 15% of the samples at slaughterhouse 1, 29.1% at No. 2 and 43.3% at No. 3 registered a coliform bacteria count lower than 3 CFU/g.

There were differences in the proportion of types of microorganisms, for example the number of the coliforms appeared to be lower at slaughterhouse 1, 80.8% of the samples showed a count lower than  $10^3$  CFU/g, with respect to 93.3 % of the other two slaughterhouses. This difference was reflected also in *E. coli* count.

Group D streptococci tended to occur in a larger proportion at slaughterhouse 2.

Table 4 - Distribution of Enterobacteriaceae at the three slaughterhouses

Slaughterhouses		1		2		3	
N° of the samples		117		118		110	
		freq.	%	freq.	%	freq.	%
range							
from to below							
(Log CFU/g)							
0.48	2	22	18.8	37	31.3	39	35.4
2	3	50	42.7	40	33.8	47	42.7
3	4	34	29	31	26.2	16	14.5
4	5	10	8.5	10	8.4	7	6.3
5	6	1	0.8			1	0.9

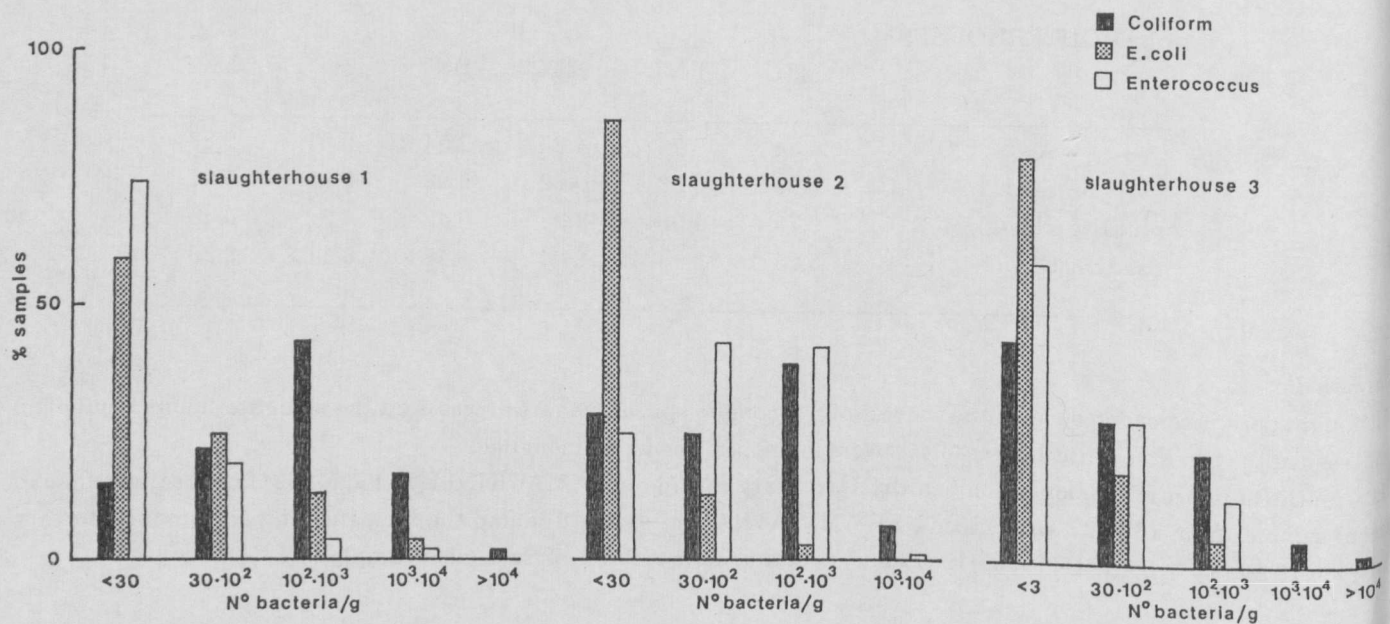
#### Identification of Enterobacteriaceae

The results obtained demonstrated the presence of a wide variety of species of Enterobacteriaceae in carcasses. The most frequently occurring were, apart from *E. coli*, *E. agglomerans*, *K. oxytoca*, *S. liquefaciens* and *C. freundii*.

Both faecal and environmental contamination may be regarded as a source of these species.

There was no difference in microbial populations between the three slaughterhouses visited, with the exception of *Enterobacter gergoviae* that was found only at slaughterhouse 2.

Fig. 1 - Coliforms, *E. coli* and *Enterococcus* from slaughterhouses 1, 2 and 3.



## CONCLUSIONS

There was a great variability in the total bacterial counts among carcasses also from the same slaughterhouse, whereas no significant differences were observed between the four sites on a carcass. This observation was reflected also in *Enterobacteriaceae* counts. The presence of coliforms and *E. coli* is rather significant because it indicates the likelihood of faecal contamination during slaughter. The levels of coliforms were generally low.

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