Psychrotroph contamination of pig carcasses

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The spoilage microflora of meat and carcasses held at chill temperatures comprises psychrotrophs and under moist aerobic conditions pseudomonads often predominate (Gill and Newton, 1978). Psychrotrophic pseudomonads also comprise a proportion of the total aerobic microflora isolated from meat and carcass surfaces by cultural methods at incubation temperatures of 25° - 30°, this proportion depending on various factors including the temperature of storage. Their determination on chilled meat and carcasses is of importance with respect to the keeping quality of the meat and derived products.

During the last few years increased interest has been shown in the establishment of microbiological standards for meat (Johnston, 1975) and numerous national and international bodies are actively considering the implementation of such standards. Currently the main priorities lie in the securing of reliable and acceptable sampling techniques and methods for the enumeration of micro-organisms (Shewan, 1976). While the implementation of standards faces many problems there is a general consensus that the control of microbial contamination of meat carcasses is relevent in terms of keeping quality of meat and for reasons of public health.

Many of the existing analytical procedures for the evaluation of meat spoilage relate to the physical or chemical assessment of terminal changes (Turner, 1960). Reliable and rapid methods for the estimation of the surface microflora of meat are not available and it is apparent that on a modern slaughter line there is little time for elaborate sampling procedures. Sampling methods for carcasses require to be non-destructive, simple, reproducible and economical. Vanderzant et al (1976) critically reviewed available sampling procedures and analytical techniques.

Methods of enumeration of bacteria recovered from meat surfaces generally involve traditional cultural procedures which require the provision of specialised laboratory facilities. Costs, particularly with respect to consumable materials, are high and the system requires extensive scientific and technical support.

Cultural evaluation of samples involves a necessary time-lag between sampling and the provision of results which, produced 2 - 10 days after sampling, may only be of retrospective significance relative to the carcasses from which they were derived. To date there have been few methods developed for the rapid evaluation of the microbiological status of meat or carcass surfaces. Scholefield et al (1976) described the application of fluorescence microscopy for the direct enumeration of bacteria in milk and urine samples and in meat washings.

The objectives of the present study were to compare certain sampling procedures in an abattoir situation and to apply a selected procedure for the recovery of total aerobic and psychrotrophic bacteria from pig carcass surfaces. Changes in the microflora of carcasses were examined and a comparison was made of the total aerobes and psychrotrophs as determined by standard cultural examination and by a rapid method based on direct fluorescence microscopy (DFM).

Materials and Methods

a) Sampling of carcasses

Pig carcasses being processed on the slaughter line of Glasgow District Abattoir were sampled using the following procedures without interference in the normal line handling.

i) Single dry cotton swab. An aluminium template (2.5cm^2) was flamed in ethanol, placed on the sample site and sterile 2.5 x 17 cm swab applied to the test area with a circular motion, rotating to ensure uniform contact. The swab was then aseptically broken into a universal bottle containing 10 ml. sterile Ringers solution.

ii) Single wet alginate swab. The procedure as described in i) but wetting the alginate swab with Ringers solution before application and breaking the used swab into 10 ml "Calgon" Ringers solution in a universal bottle.

iii) Double wet/dry cotton swab. The procedure as described for i) with the initial application of a moist swab to the test area followed by a dry swab to the same area, combining the two swabs in a universal bottle containing Ringers solution.

iv) Excision. Using sterile scissors and forceps a portion of the skin was aseptically removed from the carcase and transfered to a sterile universal bottle.

v) Rinsing and scraping. The method was adapted from that described by Williams (1967).

All samples were transported to the laboratory under chill and prepared for examination within two hours of sampling.

b) Cultural examination

Samples were well mixed using a Rotamixer (Hook and Tucker, London), dilutions prepared and 0.1 ml aliquots were surface plated on to plate count agar (Oxoid Ltd., London), duplicate plates being incubated at 30° for 48 hours for Total Aerobe Counts and at 5° for 10 days for

Psychrotroph Counts. Excision samples required blending using a Colworth Stomacher (A.J. Seward, London)

c) Direct Fluorescence Microscopy (DFM). The system employed for the quantitative examination of preparations by DFM and described by Scholefield et al (1976) has been modified (RMG system) by the incorporation of the following components.

i) Microscope with xenon (XBO50) incident illumination (Dialux/SM Lux; E. Leitz Ltd., London), ii) Video camera (HV165 Hitachi. Japan) iii) Automatic scanning stage (Martz-Hauser, Microinstruments, Ltd., U.K.), iv) Image counting system (Optomax OM1, Micromeasurements Ltd., U.K.) and v) 9" Video monitor (Hitachi, Japan).

For the enumeration of bacterial cells a X40/0.85 fluorite objective was employed together with excitation and barrier filters and dichroic mirrors appropriate to the fluorchrome staining procedure (Scholefield et al, 1976).

Results

a) Sampling of pig carcasses.

Following dehairing, evisceration, washing and inspection 12 carcasses, which comprised one in 5 or 10 being sequentially processed, were sampled prior to chill storage. The hind limb, shoulder and pelvic and thoracic locations were sampled using the single dry swab and the double wet-dry swab techniques and the recovery of total aerobes and psychrotrophs determined culturally.

Table 1. Recoveries of total aerobes and psychrotrophs from several locations on 12 carcasses by two sampling methods.

		Hind limb		Shoulder		Pelvic		Thoracic	
Method		m	SD	m	SD	m	SD	m	SD
Single dry cotton swab	T	3.07	0.73	3.93	0.34	2.57	0.69	0.63	0.26
	P	1.81	0.41	2.16	0.28	1.49	0.55	1.49	0.21
Double wet-dry swab	T	4.04	0.20	4.60	0.16	3.58	0.20	3.21	0.41
	P	2.23	0.16	2.52	0.24	2.10	0.31	2.06	0.36

m - mean value
SD - standard deviation
T - total aerobe count
P - psychrotrophic count
Counts as $\log_{10}/cm^2$

The results shown in Table 1 indicate that the shoulder region provided the highest mean total aerobe and psychrotroph counts with least variation overall. The double wet-dry swab was superior to the single dry swab in the recovery of bacteria at all locations, this being particularly evident for the shoulder region.

b) Comparison of sampling techniques.

Sampling procedures including i) single dry swab ii) single wet alginate swab iii) double wet-dry swab iii) excision and iv) rinse - scrape were applied to the shoulder region of 36 carcasses prior to chill storage. Total aerobes and psychrotrophs were culturally determined.

Table 2. Recoveries of total aerobes and psychrotrophs from the shoulder region of 36 carcasses by five methods

Method	Single dry cotton swab		Single wet alginate swab		Double wet-dry cotton swab		Excision		Rinse-scrape	
Count	m	SD	m	SD	m	SD	m	SD	m	SD
Total aerobes	3.82	0.42	4.32	0.26	4.76	0.18	5.41	0.95	5.25	0.80
Psychrotrophs	1.46	0.32	2.06	0.20	2.64	0.28	2.86	0.72	2.78	0.76

Excision and rinse-scraping gave the highest mean recoveries of total aerobes and psychrotrophs although the variation between results was great. The results confirmed that the double swab method gave good recovery of both total aerobes and psychrotrophs and, compared with the rinse-scrape method, was rapid and convenient in use on a slaughter line. Regression analysis of total aerobe and psychrotroph counts from the 36 carcasses by the double swab method indicated no correlation between the two sets of results.

To determine the relationship between level of contamination and recovery sterile samples of pig skin and meat surfaces were inoculated with 18 hours cultures of Ps. fluorescens grown at 25° to provide levels of $\log_{10}$ 3.0 - 5.0 per cm^2. Skin or meat samples were incubated at 5° or 10° and sampled at intervals using the excision and double swab method followed by cultural enumeration.

A positive correlation was found (r = 0.979) between the level of contamination and the efficiency of recovery of Ps. fluorescens from either skin or meat surfaces, ranging from 62% recovery at $\log_{10}$ 4.0 to 95% recovery at $\log_{10}$ 9.0 per cm^2.

c) Changes in microflora of carcasses during processing. The double swab technique was applied to 36 pig carcasses at three sampling points. The results shown in Table 3 indicate the reductive effect of scalding on the psychrotroph population. However, recontamination during evisceration served to increase levels of total aerobes and psychrotrophs on the dressed carcasses.

d) Microbiological changes on chilled carcasses.

Carcasses were sampled from shoulder region using the standard procedure at the pre-chill inspection point and then at intervals during storage at 3.3° for up to eight days. Total aerobes and psychrotrophs were culturally determined.

Table 3. Recovery of total aerobes (T) and psychrotrophs (P) from 36 carcasses at three stages in process.

	T		P	
	m	SD	m	SD
After bleeding	4.73	0.22	4.15	0.38
After dehairing	4.24	0.46	1.73	0.65
Before chilling	4.76	0.18	2.64	0.28

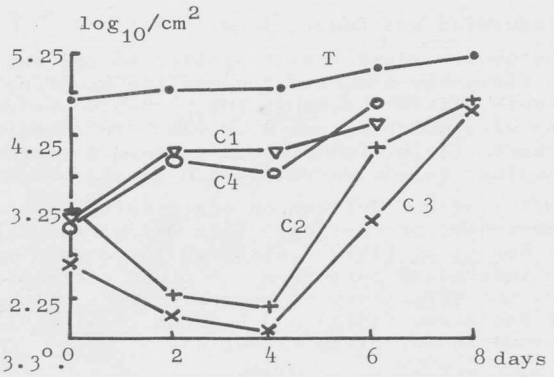


Figure 1. Total aerobe (T) and psychrotroph (P1-P4) counts on carcasses at 3.3°.

The results demonstrate a progressive increase in total aerobes on carcasses at 3.3°, of which an increasing proportion comprised psychrotrophic bacteria. The pattern of psychrotroph growth varied, with progressive increases on carcasses C1 and C4 and on initial lag in growth being demonstrated on carcasses C2 and C3. (Figure 1).

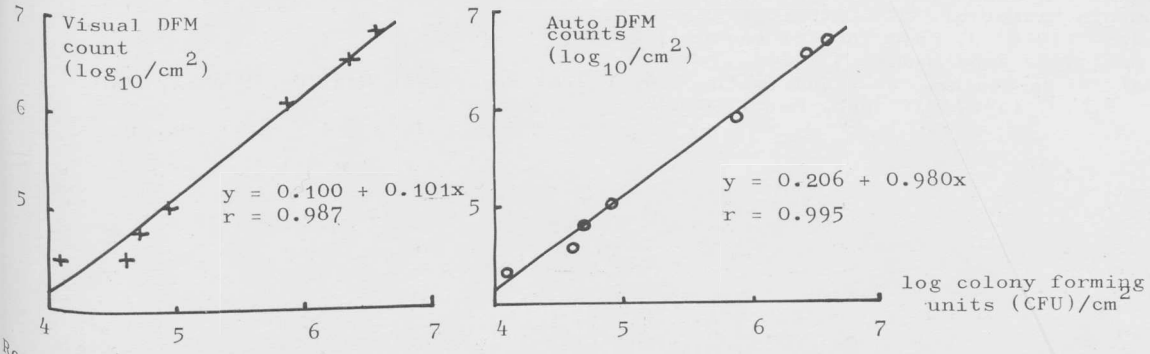
e)

Assessment of carcasses by Direct Fluorescence Microscopy.

Samples of pig skin were sterilised by treatment with 10% (w/v) sodium hypochlorite solution for 15 minutes followed by neutralisation of excess chlorine with 10% (w/v) sterile thio - sulphate. Ringers solution and rinsing with sterile Ringers solution. After sterility checks 2.5 cm^2 areas of samples contained in sterile petri dishes were inoculated by micro-pipette with an 18 hour culture of Ps.fluorescens to provide $\log_{10}$ 3-5 cells per cm^2. The standard double swab method was employed to recover bacteria from the test samples held for 10 days at 5°. Rotamixed samples were filtered* and 2 µl aliquots of the filtrate spread over an etched 10 mm area of cleaned microscope slides. After drying, fixation in 50:50 ethanol: acetic acid was followed by staining with 0.05% (w/v) acridine orange (G.T. Gurr, London) in 0.07M H_3BO_3 - $NaH_2PO_4 2H_2O$ buffer at pH 5.5 for 5 minutes at 20°. Preparations were washed in cold water, dried at 20° and examined by DFM. For each preparation an accumulated number of fluorescing bacteria or groups of bacteria were enumerated on a randomised field basis to obtain a 90% confidence interval (Cassell, 1965). Enumeration was performed visually, and automatically with the Optomax counting unit. Total aerobe and psychrotroph counts were made in parallel with DFM counts.

(*100-120 µ Sintered glass filter).

Figure 2. Regression analysis of cultural and DFM counts of Ps. fluorescens on pig skin



Regression analysis (Figure 2) indicated good correlation between DFM and cultural counts, particularly above $\log_{10}$ 5.0/cm, although visual DFM counts tended to be higher than cultural counts. By adjustment of selector controls on the Optomax unit the numbers of cells enumerated may be regulated on the basis of visual fluorescence level. By this procedure auto DFM counts were found to exhibit an improved relationship with cultural counts, particularly below $\log_{10}$ 5.0/cm^2.

Auto DFM, total aerobe and psychrotroph counts were performed on double swab samples from 7 pre-chill carcasses and on 5 carcasses held for 5 days at 3.3°.

Table3. Regression analysis of cultural and auto-DFM evaluation of carcasses.

Count	Auto-DFM pre-chill carcasses (7)	Chilled carcasses (5)
Total aerobe	y = 3.50 + 0.46x r = 0.737	y = 5.27 + 0.17x r = 0.685
Psychrotroph	y = 4.98 + 0.23x r = 0.457	y = 5.28 + 0.19x r = 0.685

The results show that for unchilled carcasses a fair correlation existed between total aerobe and DFM counts. After chill storage correlation between psychrotroph and DFM counts improved. These observations are supported by the findings that psychrotrophs comprise an increased proportion of the total aerobes on chill stored carcasses.

Discussion and Conclusions.

The double wet-dry swab applied to the shoulder region was found to be reproducible and sufficiently accurate for on-line sampling of the microflora of pig carcasses. Total aerobic counts within the range $\log_{10} 4.0\text{-}5.0$ per cm^2 were isolated at stages from slaughter to chill storage, which agrees with the level obtained by Kitchell et al (1973). Whereas Ingram and Roberts (1976) found a non-uniform distribution of bacteria this study indicated that the shoulder region carried the highest contamination on uncut carcass surfaces.

Following a reduction on scalding the proportion of psychrotrophs tended to increase during subsequent processing. This was particularly apparent during chill storage, as also observed by Rey et al (1970), although there were marked variations in the development of psychrotrophs on individual carcasses. A clear correlation was shown between the level of contamination and the efficiency of recovery of Ps. fluorescens from pig skin, which confirms the findings of Patterson (1971) and Lazarus et al (1977). This factor may affect the choice of sampling method in any given situation.

Direct Fluorescence Microscopy counts were found to correlate well with total aerobic counts of carcasses sampled prior to chill storage. The best relationship with psychrotrophic counts was established for carcasses which had been chill stored prior to distribution. It is apparent that the wider implementation of microbiological standards for meat and carcasses, as highlighted by Christian (1972), is to a great extent governed by the economic constraints of traditional cultural procedures. Therefore, combined with a practical sampling procedure, such as double wet-dry swabbing, the DFM system represents a possible alternative to cultural procedures for the rapid economical assessment of the microbiological status of pig carcasses.

Continuing studies relate to the application of DFM methods to the rapid quantitative and qualitative assessment of microflora of spoilage and public health significance on meat, carcasses and food content surfaces.

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