

Studies on the estimation of the hygienic condition of frozen broiler chickens

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SUMMARY

Various sampling techniques to determine the aerobic, *E. coli* and Enterobacteriaceae counts and to detect the presence of salmonellas were compared. As a simple method for the detection of salmonellas a modified Surkeiwicz procedure using both drip and rinse water is advocated. To evaluate hygiene during processing, determination of the number of Enterobacteriaceae in pieces of skin from the ventral, lateral and breast region is preferred.

INTRODUCTION

Many methods have been elaborated to determine the bacteriological condition of poultry carcasses, but the sampling techniques differ widely and moreover there is a wide variety of significant organisms (Barnes, Impey & Parry, 1973). It is not easy to make a choice since the advantages or disadvantages, if any, are not always clear. We compared several sampling and enumeration methods, which were chosen from the public health point of view, to select a method for the estimation of cleanliness during processing and to elaborate a procedure for the isolation of salmonellas. Furthermore the use of some methods more than others would enable the findings to be compared with the results of other workers.

MATERIALS AND METHODS

Frozen broilers of *ca.* 1000 g. from five different processing plants were obtained from shops in the neighbourhood of the Institute. On receipt at the laboratory the plastic covers were removed and the carcasses packed in a new plastic bag. After 48 hr. thawing at 4° C. the chickens were examined in different ways.

From the first series of chickens the drip fluid was removed and with sterile instruments pieces of skin were taken from the breast (*ca.* 2-3 g.), the pericloacal region (*ca.* 2-3 g.) and the neck (*ca.* 10 g.). The carcass was then shaken 15 times in 1 l. of sterile distilled water (Leistner & Szentkuti, 1970). This rinse water and the

drip fluid were used for the isolation of salmonellas only. The pieces of skin were used to isolate salmonellas as well as to determine the Enterobacteriaceae and aerobic counts. Finally the carcass was minced in a meat grinder (Leistner & Szentkuti, 1970) and the minced meat was used for the isolation of salmonellas.

To the second series of chickens a volume of buffered peptone water (BPw) (Van Schothorst & Van Leusden, 1972) equal to half the weight of the chickens was added to the bag containing the drip fluid and the carcass (Surkeiwicz, Johnston, Moran & Krumm, 1969), 30 ml. of a 5% Tergitol solution was added per litre of BPw. After shaking for 1 min. the mixture of rinse and drip fluid was used for the isolation of salmonellas and also to determine the aerobic, Enterobacteriaceae and *Escherichia coli* counts. After rinsing the carcass, pieces of skin were taken from the breast, pericloacal region (vent) and neck. Those from the vent and the breast were used for the isolation of salmonellas only. The piece of neck skin was also used to determine the various counts. The chickens were then minced and the meat examined for salmonellas.

The various samples were examined for salmonellas by the following procedures. (i) The drip fluid (first series) was added to 9 times its volume of BPw. (ii) The rinse water (first series) was added to 250 ml. of 5 times normal strength BPw. (iii) The rinse water containing the drip fluid in the second series was used directly as the pre-enrichment medium. (iv) The pieces of skin (first and second series) were individually blended with 9 times their weight of physiological saline containing 0.1% peptone (pfs) (Büchli, Van Schothorst & Kampelmacher, 1966), 10 ml. portions of the macerated material were transferred to 90 ml. vols. of BPw. (v) 25 g. (first series) or 50 g. (second series) of the minced meat were added to 250 or 500 ml. of BPw respectively.

All the BPw pre-enrichment cultures were incubated for 18–20 hr. at 37° C. After incubation 10 ml. portions were transferred to 100 ml. vols. of tetrathionate bile brilliant green liquid enrichment medium (TBB). After 24 hr. and 48 hr. incubation at 43° C. the enrichment cultures were streaked on brilliant green phenol red agar plates (BGA). The plates were read after 18–24 hr. incubation at 37° C. and suspect colonies were confirmed as salmonellas using triple sugar iron agar and lysine decarboxylase medium and serotyped (Edel & Kampelmacher, 1973).

To determine the aerobic, Enterobacteriaceae and *E. coli* counts decimal dilutions were prepared from the fluids or the macerated material. Aerobic counts were made in or on Tryptone–dextrose–yeast extract–milkpowder agar (TDYM, Mossel & Krugers Dagneaux, 1959) by both the pour plate method (all chickens) and the surface plate method by spreading 0.1 ml. (27 chickens of the second series). Colonies were counted after 3 days incubation at 30° C. or 5 days at 21° C. (second series). The Enterobacteriaceae count was determined on violet red bile glucose agar (VRBG) using the pour plate method with overlay (Mossel, Mengerink & Scholts, 1962) and the surface plate method (26 chickens of the second series). In the surface plate method only particular colonies (rose red with halo) were counted. When the pour plate method was used all rose red colonies were counted as Enterobacteriaceae; no further identification tests were carried out. The plates

Table 1. *Salmonella* isolations from frozen broilers

	First series	Second series
Number examined	46	53
Number of positive samples via:		
Neck skin	23	27
Breast skin	15	13
Pericloacal skin	17	20
Drip water	16	34
Rinse water	18	
Minced carcass meat	20*	30†
Total positive isolations (all methods)	34 (73.9%)	39 (73.6%)

* 25 g. examined.

† 50 g. examined.

were incubated at 37 or at 31° C. (second series) for 20–24 hr. To determine the number of *E. coli* present (all chickens of the second series) the MPN procedure (3 replicates) with brilliant green bile lactose medium (BGBL) was chosen. After 48 hr. incubation at 31° C. the tubes were examined for gas production. From positive tubes 0.5 ml. vol. were transferred to fresh tubes of BGBL and tryptone water. After 24 hr. incubation at 44 ± 1° C. the tubes were examined for gas and indole production.

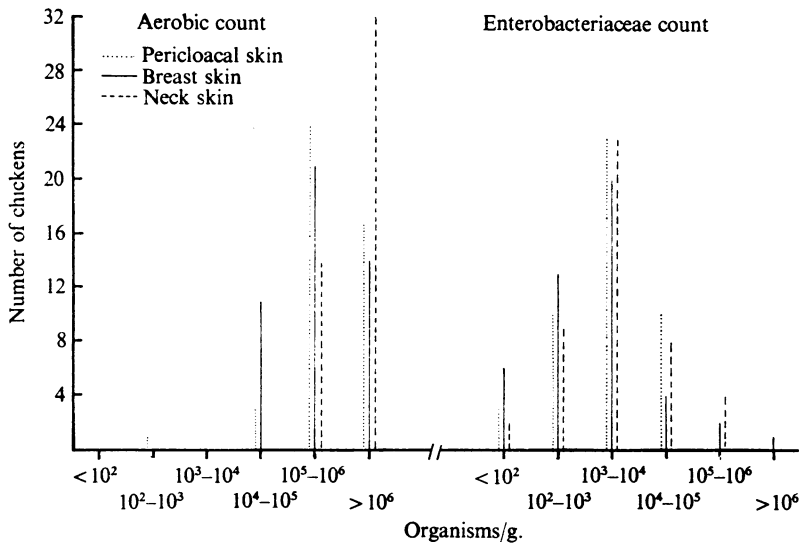


Fig. 1. Aerobic and Enterobacteriaceae counts of various pieces of chicken skin (first series).

RESULTS

In the first series 46 chickens were examined. Salmonellas were isolated from 34 by one or more of the six methods employed. In Table 1 the number of isolations by the different methods are tabulated. The highest number of positive samples was obtained by examining the neck skin and the lowest by examining the breast skin. The aerobic and Enterobacteriaceae counts are given in Fig. 1.

Table 2. *Frequency of E. coli counts in 53 frozen chickens (2nd series)*

	MPN per ml. or g.		
	< 10 ³	10 ² -10 ³	- 10 ³
Rinse-drip water	48	4	1
Neck skin	6	27	20

The highest aerobic counts were found on the neck skin and the lowest on the breast skin. This difference is significant at the 95% level. The differences in *Enterobacteriaceae* counts are less obvious. The logarithmic mean of the counts and the standard deviations were: pericloacal skin 3.4 ± 0.8 , breast skin 3.1 ± 0.9 and neck

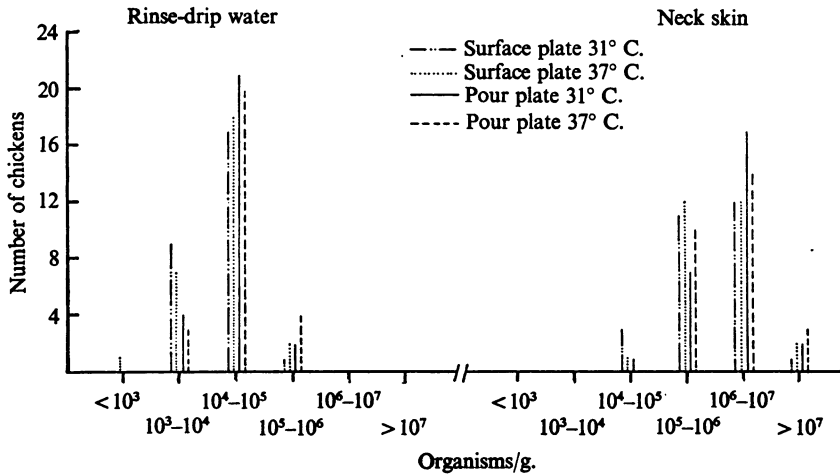


Fig. 2. Aerobic counts of rinse-drip water and pieces of neck skin using various counting methods (second series of chickens).

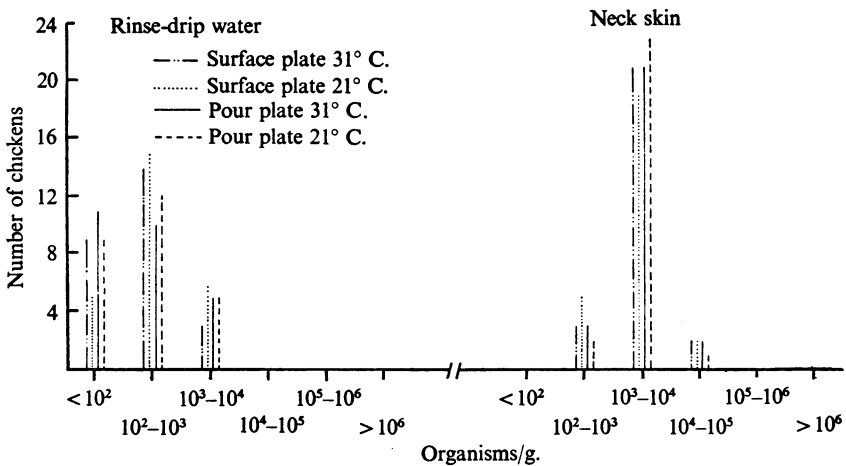


Fig. 3. *Enterobacteriaceae* counts of rinse-drip water and pieces of neck skin using various counting methods (second series of chickens).

skin 3.5 ± 0.9 . By means of the rank correlation test of Spearman a correlation between the three counts on one carcass could be demonstrated (with a 99% probability). The same was true for the three aerobic counts.

In the second series 53 chickens were examined, salmonellas were isolated from 39 birds by one or more of these methods (Table 1). The *E. coli* counts are presented in Table 2 and the frequency distribution of the aerobic and Enterobacteriaceae counts obtained by the various methods is presented in Figs. 2 and 3. After comparing the Enterobacteriaceae counts, obtained with the different methods, by means of the sign test, it was concluded that there were no significant differences between the counts. The aerobic pour plate counts, for rinse drip water at 21° C. and neck skin at 21 and at 31° C., were somewhat (log 0.3) higher than those obtained by the surface-plate method. There were no significant differences between the aerobic counts for rinse-drip water at 31° C. There was no correlation between the counts from neck skin and rinse water.

DISCUSSION

These studies were carried out for two reasons: to study the bacteriological condition of frozen Dutch chickens and to study the methods for determining this condition. Table 1 clearly demonstrates that the assessment of bacteriological condition may be strongly influenced by the method used. The contamination rate of the chickens with salmonellas was 25–70%, depending on the methods used.

In choosing a method the significance of these figures in relation to public health is of great importance. The organisms which are within the chickens, or firmly attached to the skin (Notermans & Kampelmacher, 1974), will probably not contaminate the kitchen and will normally be killed during cooking (Roberts, 1972). It can therefore be postulated that those salmonellas which are in the drip water, or which can be washed off easily, are most likely to present a public health hazard by contaminating the kitchen, and thus contaminating birds after they are cooked. It therefore seems reasonable to subject frozen chickens to a sampling treatment such that it will detect those organisms that are likely to give rise to such contamination. For this reason the use of rinse-drip water for the examination of chickens for the presence of salmonellas seems to be advisable.

With regard to the determination of the aerobic, Enterobacteriaceae and *E. coli* counts, the choice between the use of the skin-blending method or the rinsing method has to be made on different grounds. These counts should be indicative of the efficiency of hygiene during processing. In these studies no correlation could, however, be found between the counts obtained with the two methods. This could mean that examination of the rinse-drip water gives a different measurement of the hygiene during slaughtering than the skin-blending method. It could well be, for instance, that with the rinsing method the results reflect only the hygienic precautions taken at the end of the slaughter line, e.g. washing and spinchilling, while the skin-blending method reveals the build up of micro-organisms on the skin throughout the whole slaughter process. The studies of Notermans &

Kampelmacher (1975) on the attachment and detachment of bacteria to the skin are pointing in this direction. If this line of reasoning is right, then the skin-blending method is more efficient. Taking into account the fact that the neck skin can be removed easily during processing, the use of this piece of skin is undesirable. To control proper handling of the carcasses during all slaughter procedures, the examination of pieces of skin from the breast, ventral and lateral regions, would perhaps be better. As a group of organisms, the Enterobacteriaceae or *E. coli* are to be preferred as indicators of poor hygiene, since the aerobic count of raw products often indicates the shelf-life rather than the safety of the products (Mead & Thomas, 1973). From a practical point of view the enumeration of Enterobacteriaceae with pour or surface plates is to be preferred since the number of *E. coli* is determined by the MPN method. It is well known that wide variations are obtained with the MPN method and therefore plating methods should be used instead, whenever possible. For Enterobacteriaceae counts, pour plates with overlay incubated at 37° C. are recommended, since virtually no organisms belonging to other taxonomic groups are detected and identification of colonies is unnecessary (unpublished results). Not all our data on the bacteriological condition of frozen broilers can be compared with those of other workers, since there were often minor differences in techniques. The aerobic and Enterobacteriaceae counts of rinse-drip water may, however, be compared with the standard counts suggested by Leistner, Woltersdorf & Melko (1973). More than 90% of the broilers examined could meet these specifications for spinchilled broilers, but further studies are required to evaluate the usefulness and reliability of such standards to guarantee hygienic conditions during processing.

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