

DETECTION OF *LISTERIA* SPECIES IN REFRIGERATED CHICKEN CARCASSES USING CLEARVIEW™ AND A MODIFIED CONVENTIONAL CULTURE METHOD

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ABSTRACT

The occurrence of *Listeria* species in refrigerated chicken carcasses was evaluated, comparing the conventional culture methodology of FDA, modified by the introduction of a secondary enrichment step prior plating, and the Clearview™ rapid method (Oxoid, UK Ltd). Forty-eight refrigerated whole chicken carcasses purchased from supermarkets in Florianópolis, SC, Brazil, were analysed. *Listeria* species occurred in 21 (43.7%) samples. Using the Clearview method, 17 (35.4%) samples were positive for *Listeria* species. Of these isolates, 11 (23%) were *L. monocytogenes*, 4 (8.3%) *L. innocua*, 1 (2.1%) *L. welshimeri* and 1 (2.1%) *L. seeligeri*. Using the conventional culture methodology of FDA (with modifications), 14 (29.2%) samples were positive for *Listeria* species. Among these 7 (14.6%) were *L. monocytogenes*, 6 (12.5%) *L. innocua* and 1 (2.1%) *L. seeligeri*. With the Clearview rapid method plus API Listeria for identification, results were confirmed to *Listeria* species level within 115-139 h. Using the conventional culture method of FDA (with modifications) plus API Listeria, results were confirmed within 120-160 h. However, the Clearview method could indicate the presence of *Listeria* organisms in only 43 h. Results given by the methods were in moderate concordance and the differences between them were not significant (C.I. = 95%).

Key words: *Listeria*, chicken carcasses, Clearview™

INTRODUCTION

Listeria monocytogenes has been recognised as a pathogen for more than sixty years. However, it has only been identified as a foodborne pathogen after the 1980s, as a result of many human outbreaks of listeriosis (7,15).

L. monocytogenes is widely spread in nature, being commonly found in natural food products and food processing environment as a biofilm which has the capacity to multiply at refrigeration temperatures (10). The organism has been isolated from soil, silage, vegetation, faecal material, water and domestic and industrial effluents (9). Men, animals and the environment are natural reservoirs for this organism. It has been isolated from a variety of animals: more than forty species of mammals and a minimum of 17 different species of birds, including domestic chicken and turkey (4).

Many foods have been implicated in outbreaks of listeriosis including raw and pasteurized milk, cheese, raw and cooked meat products from different animals, vegetables, fish products and ready to eat foods (15). The contamination of both frozen and refrigerated chicken is high in comparison to other foods (13). According to Uboldi Eiroa (16) and Uyttendaele *et al.* (17), this high incidence is alarming and increases the risk for cross-contamination between raw and cooked food during preparation. It is therefore important to devise a good quality control programme for this bacterium when processing chicken products.

Recent foodborne outbreaks of listeriosis stress the need to develop effective methods for the detection and identification of *L. monocytogenes*. Particularly, it is important to develop sensitive and rapid methods since conventional methods are time-consuming and laborious.

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This study investigated the occurrence of *Listeria* species in refrigerated whole chicken carcasses sold in supermarkets in Florianópolis, SC, Brazil and evaluated the Clearview™ rapid method (Oxoid, UK Ltd.) (11) in comparison to the modified FDA conventional culture methods (5,6) for detection of *Listeria* species. Clearview™ rapid method is based on the immunoassay technology for the detection of *Listeria* flagella antigen in cultured samples using monoclonal antibodies. Flagellar protein is extracted from *Listeria* cells and added to a membrane to which a line of anti-flagellin antibody is bound. If bound flagellin protein is present, it is detected by the addition of a second soluble, anti-flagellin antibody, blue latex complex. The development of a blue line indicates the presence of *Listeria* species flagellin protein (11).

MATERIALS AND METHODS

Collection, preparation of samples for analysis and testing

Forty-eight refrigerated whole chicken carcasses were randomly collected from six supermarkets in Florianópolis, SC, Brazil, from May to July 1999. Samples were transported to the laboratory in a cold box filled with ice and subjected to microbiological analysis within one hour of collection.

The carcasses were removed aseptically from their original packaging and repackaged in sterile plastic sample bags. Buffered peptone water (BPW) (300 ml) was added to each bag and shaken fifty times to wash the carcass evenly. The BPW was then poured off into a sterile measuring cylinder.

Conventional culture method of isolating *Listeria* species was modified by the introduction of secondary enrichment step prior plating. A 25 ml aliquot of BPW was added to 225 ml of Listeria Enrichment Broth – LEB (Oxoid, UK). The samples were incubated for 4 h at 30°C, then selective supplements (SR 140E, Oxoid, UK) were added, and samples were incubated a further 20 h. This step helps the recovery of stressed *Listeria* cells from the food samples (5). Following incubation, 0.1 ml of LEB was transferred to 10 ml Fraser Broth (FB) and incubated at 30°C for 24 to 40 h. A loopfull of the broth was plated on PALCAM agar (Oxoid, UK) and incubated at 35°C for up to 48 h. Three typical single colonies were streaked onto Tryptone Soya Yeast Extract Agar (TSYEA) (Oxoid, UK, Ltd.), incubated at 35°C for 24 h, and submitted to biochemical identification to *Listeria* species.

Clearview™ method also employs two enrichment steps to selectively grow *Listeria* organisms. 25 ml of BPW was added to 225 ml of FB and incubated at 30°C for 21 h. Following incubation, 0.1 ml of FB was transferred to 10 ml LEB and incubated at 30°C for 21 h. An aliquot (2 ml) of LEB was heated at 80°C for 20 min in a water bath to release flagellin protein. The aliquot was then cooled to room temperature and 135 ml inoculated onto the Clearview™ immunoassay strip as directed by the manufacturer. The results were read after 20 min incubation at room temperature. Positive

samples were identified to *Listeria* species level by plating a loopfull of unheated LEB onto PALCAM agar (Oxoid Ltd. UK) which was incubated at 35°C for up to 48 h. Typical single colonies were streaked onto Tryptone Soya Yeast Extract Agar (TSYEA), incubated at 35°C for 24 h, and submitted to biochemical identification.

Identification of isolated colonies to the species level from both the modified FDA conventional culture and the Clearview methods was done using the API Listeria identification strips (10300, bioMérieux, Marcy-l'Étoile, France).

Evaluation of methodologies

The sensitivity of a method is related to its capacity to avoid false negative results, while specificity is related to its ability to not produce false positive results. In this study, a sample was considered true positive when presumptive positive results in both methods were confirmed as *Listeria* species by the API Listeria identification strips. A sample was considered a true negative when it was negative by the two methods or when it was presumptive positive by one method, but failed to confirm the presence of *Listeria* species. A result was considered a false positive when it was positive in the kit but was not confirmed as *Listeria*. A sample was considered as a false negative when it showed a negative result by one method but was a confirmed positive in the other.

Statistical analysis of data

Two non-parametric statistical tests were applied to the data. The first test used SAS software (Microsoft, USA) and determined the coefficient of concordance between the two methods with a confidence interval (CI) of 95% and a α of 5%. The second test correlated the results obtained by the two methods using Statgraf software (Microsoft, USA) with a CI of 95%.

RESULTS

Samples of chicken carcasses were considered positive if *Listeria* species were isolated by either method studied and the isolates then identified to the species level using API-Listeria strips. Combining the results of the two methods, it was found that 21 out of 48 (43.7%) refrigerated chicken carcass samples were *Listeria* positive.

The total number of samples positive for *Listeria* species using the modified FDA conventional culture method was 14 (29.2%). Four (8.4%) of these samples were negative by the Clearview rapid method (Fig. 1). The total number of samples positive for *Listeria* species using the Clearview rapid method was 17 (35.4%) and seven (14.6%) of them were negative by the conventional culture method (Fig. 1). The number of samples found positive by both methods was 10 (20.8%).

Among 17 samples positive for *Listeria* species by the Clearview method, 11 (23%) were *L. monocytogenes*, 4 (8.3%) *L.*

innocua, 1 (2.1%) *L. welshimeri* and 1 (2.1%) *L. seeligeri*. Of the 14 samples positive for *Listeria* species by the conventional culture method; 7 (14.6%) were *L. monocytogenes*, 6 (12.5%) *L. innocua* and 1 (2.1%) *L. seeligeri* (Fig. 2). None of the samples yielded more than one *Listeria* species.

The rapid Clearview™ method identified samples positive for *Listeria* in 43 h. By the modified FDA conventional culture method,

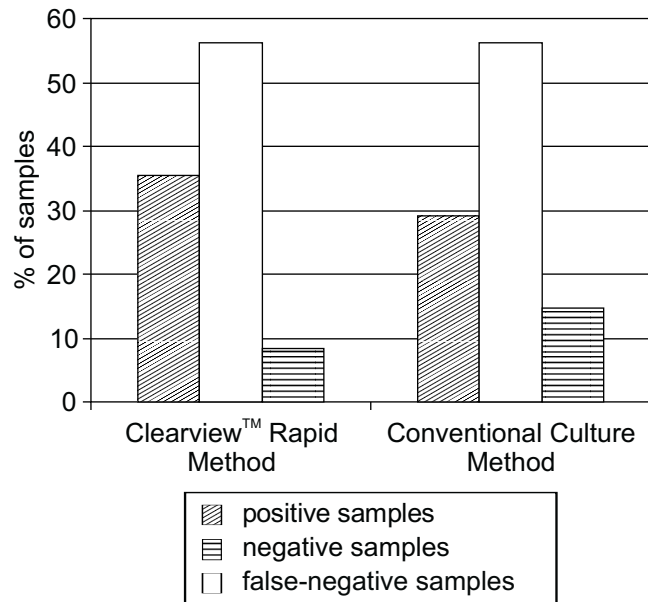


Figure 1. Comparison of the modified FDA conventional culture and the Clearview™ methods for the detection of *Listeria* species in refrigerated chicken carcasses.

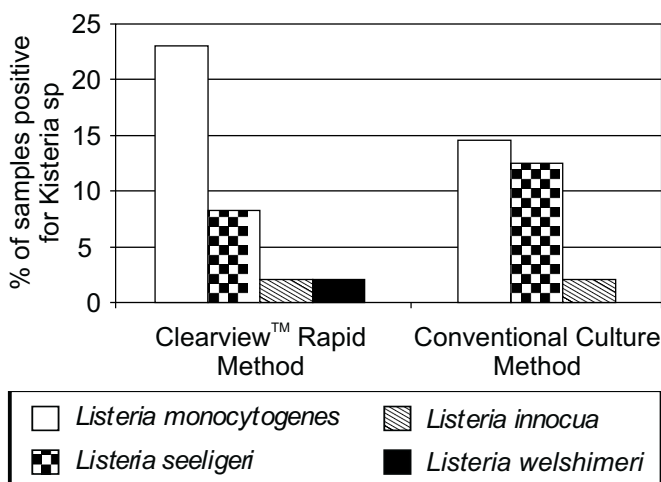


Figure 2. Percentage of chicken carcass samples positive for *Listeria* species using the Clearview and the modified FDA conventional culture methods.

presumptive *Listeria* colonies were detected after 72 to 112 h, which were confirmed species level 48 h later (120 to 160 h).

Results for presence or absence of *Listeria* species observed in both methods presented a concordance coefficient of 48%. This was considered a moderate concordance, but significant for a 5% (C.I. 95%). On comparing positive and negative results, the paired correlation test showed that the two assay methods were not significantly different at a significance level of 5%.

DISCUSSION

The incidence of *Listeria* species in chicken carcasses was similar to that reported previously (1,19) using conventional culture methods of isolation. There was a variation in incidence ranging from 2 to 94% for the isolation of *Listeria* species from chicken depending upon the country of origin and the method employed for isolation (18).

The four false negative samples found by the Clearview™ rapid method could perhaps be ascribed to insufficient antigen being present for the immunological assay if there was only a small amount of growth in the prior enrichment culture step. According to Roberts (14), the Clearview™ rapid method requires a minimum level of about 5×10^4 to 1×10^5 bacteria/ml in the LEB enrichment step for the assay to record a positive result. It is suggested that the count could be assessed photometrically or confirmed retrospectively by plate counting LEB intended for immunoassay. The same author found no false positives and only one false negative (a chicken sample) were found using the Clearview™ method on 995 food samples. The seven false negative samples found by the modified FDA conventional culture method could possibly have been due to the presence of other bacteria which inhibited the growth and therefore the isolation of *Listeria* species. These false negative samples were positive in the Clearview™ rapid method. This may be because the more inhibitory FB (having more acriflavine hydrochloride) is used before LEB in the Clearview protocol which may have discouraged growth of other bacterial species.

The presence of *Listeria* species in 43.7% refrigerated chicken carcasses in Florianópolis, Brazil, suggests that there is a significant contamination problem in the processing chain. *L. monocytogenes* was the most commonly isolated species (23% and 14.6% of *Listeria* species isolated by Clearview™ rapid and modified FDA conventional culture methods, respectively). With an increased consumption of chicken in this region this occurrence poses a significant threat for an increase in foodborne listeriosis, if chicken is undercooked.

Compared to conventional culture methods, Clearview method is substantially quicker in detecting the presence of *Listeria* species in samples. By the Clearview™ rapid method. Samples can be considered negative for *Listeria* organisms after only 43 h. Thus, the method is convenient for screening *Listeria* negative food samples.

RESUMO

Detecção de *Listeria* spp em carcaças refrigeradas de frangos empregando Clearview e um método convencional de cultura modificado

Avaliou-se a ocorrência de *Listeria* spp em carcaças refrigeradas de frango, comparando-se a metodologia convencional recomendada pelo FDA, modificada pela introdução de uma segunda etapa de enriquecimento antes do plaqueamento, e o método rápido Clearview™ (Oxoid, UK, Ltd). Foram analisadas 48 carcaças de frango de diferentes marcas e supermercados de Florianópolis, Brasil. *Listeria* spp foi encontrada em 21 (43,7%) amostras. Através do método Clearview encontrou-se 17 (35,4%) amostras positivas para *Listeria* spp, das quais 11 (23%) eram *L. monocytogenes*, 4 (8,3%) *L. innocua*, 1 (2,1%) *L. welshimeri* e 1 (2,1%) *L. seeligeri*. Através do método convencional modificado obteve-se um total de 14 (29,2%) amostras positivas para *Listeria* spp, das quais 7 (14,6%) eram *L. monocytogenes*, 6 (12,5%) *L. innocua* e 1 (2,1%) *L. seeligeri*. Com o método Clearview + API *Listeria*, obteve-se resultados confirmados à nível de espécie em 115-139 h, e com o método convencional modificado + API *Listeria* os resultados foram obtidos em 120-160 h. No entanto, o método Clearview pode indicar a presença de *Listeria* spp em apenas 43 h. Os resultados obtidos pelos métodos utilizados mostraram-se moderadamente concordantes e não apresentaram diferença significativa num intervalo de confiança de 95%.

Palavras chave: *Listeria* spp, carcaças de frango, Clearview™

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