Prevalence of *Salmonella* and *Campylobacter* in Retail Chicken Carcasses in Senegal

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Key words

Chicken – *Campylobacter* – *Salmonella* – Freezing – Refrigeration – Senegal.

Summary

From January 2001 to May 2002, 300 chicken carcasses from retail shops in Dakar were examined for prevalence of *Salmonella* and *Campylobacter*. Of these, 146 were fresh products, 58 were refrigerated and 96 were frozen. *Salmonella* was isolated from 96 (32%) of the carcasses analyzed. The most prominent *Salmonella* serovars were *Salmonella* Hadar (41.6%) and *Salmonella* Brancaster (20.8%). *Campylobacter* spp. was isolated from 168 (56%) of the samples. *C. jejuni* was more frequently isolated (59%) than *C. coli* (27%). The contamination rates for *Campylobacter* were significantly different in relation to the type of carcass: 76% for fresh, 53% for chilled and 28% for frozen.

■ INTRODUCTION

Infections with *Salmonella* or *Campylobacter* are two of the most common causes of gastroenteritis worldwide. In developed countries, investigations have shown that infections caused by *Campylobacter* spp. may be as serious as those by *Salmonella* spp., both in frequency and severity of symptoms. These microorganisms are also a public health concern and a source of common complications in HIV-infected patients. Contaminated food is the usual source of human infections, and poultry products are considered the major infectious route for humans. Thus, reducing *Salmonella* and *Campylobacter* contamination of poultry products will reduce the risk of food borne disease to consumers.

To prevent chicken carcass contamination, it is important to control *Salmonella* spp. and *Campylobacter* spp. infections along the food production chain. But in spite of improved hygiene at the farm and slaughterhouse levels, numerous poultry carcasses remain infected in retail shops. Most of the reported human *Campylobacter* infection is associated with improper handling of raw chicken, eating raw or undercooked chicken and poor kitchen hygiene.

In Senegal, little is known regarding the occurrence of food borne disease caused by *Salmonella* or *Campylobacter*. For this reason, the authors decided to search for these bacteria on chicken carcasses, an increasingly consumed product. Thus, the aim of this study was to determine the prevalence of *Salmonella* and *Campylobacter* on chicken carcasses obtained from retail outlets in Dakar, capital-city of Senegal.

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■ MATERIALS AND METHODS

Samples

Between January 2001 and May 2002, 300 locally produced chicken carcasses were collected from retail outlets in Dakar. Of these, 146 were fresh, 58 were refrigerated and 96 were frozen. Chicken portions were not sampled as these were all imported. Carcasses were transported to the laboratory after being collected in a portable cooler at a temperature of 4°C, and microbiological analysis was carried out immediately.

Isolation and identification of Salmonella

Skin samples (25 g) taken from multiple points on the breast were homogenized in a stomacher lab-blender 400 (Seward, London, England) with buffered peptone water (AES Laboratoire, Combourg, France) in 1:10 sample/broth ratio at 37°C for 18–20 h. Two milliliters of this pre-enrichment broth were used to inoculate 20 ml of Müller-Kauffmann tetrathionate broth (AES Laboratoire, Combourg, France) and 100 µl of the pre-enriched broth were used to inoculate a modified semi-solid Rappaport Vassiliadis (MSRV) (Merck, Nogent sur Marne, France) agar plate. The media were incubated at 42°C and 41.5°C respectively for 24 h. Growth more than 20 mm from the point of inoculation on MSRV was streaked onto *Salmonella-Shigella* (SS) agar plates. The tetrathionate broth culture was streaked onto xylose lysine tergitol-4 (XLT4) agar plates (AES Laboratoire, Combourg, France). SS and XLT4 agar plates were incubated at 37°C for 24 h. Typical *Salmonella* colonies were confirmed by biochemical assays on Kligler Hajna medium, ONPG medium and lysine decarboxylase, then serotyped by slide agglutination test using *Salmonella* polyvalent O and H antisera (Diagnostic Pasteur, Paris, France).

Isolation and identification of Campylobacter

Bacterial strains and culture conditions

Skin samples (25 g) were added to 225 ml of Preston broth with Preston antibiotic supplement (Oxoid, England) and incubated at 42°C for 24 h under microaerophilic conditions (Campygen, Oxoid laboratory, England). Each sample was then streaked onto Vibrion plates (Mueller Hinton agar, Merck, Germany; Bacto agar, Difco laboratory, USA; with 5% of defibrinated horse blood, AES laboratory, France) and onto Karmali plates (Oxoid, England). Plates were incubated at 42°C under microaerophilic conditions for 48 h. Isolates were identified using a commercial identification method (API Campy®, bioMérieux, France) and multiplex PCR.

DNA extraction and PCR

Identification of every isolate was confirmed by a multiplex PCR, using specific primers for the *Campylobacter* genus (MD16S1, MD16S2), *C. jejuni* species (MDMapA1, MDMapA2) and *C. coli* species (COL3, MDCOL2). Briefly, *Campylobacter* spp. colonies from a blood agar plate were suspended in 0.2 ml TE buffer. Cells were lyzed by heating at 95°C for 10 min, and cellular debris was removed by centrifugation at 5000 g for 10 min. The supernatant was used as a template source for DNA amplification. Each multiplex PCR tube contained 200 µM deoxynucleoside triphosphate, 2.5 µl of 10X reaction buffer, 20 mM $MgCl_2$, 0.11 µM *Campylobacter* genus primers, 0.42 µM *C. jejuni* primers and 0.42 µM *C. coli* primers. Template DNA (3 µl) was added and the volume adjusted with sterile water to give 30 µl. DNA amplification was carried out in a Perkin-Elmer 9600® thermocycler using an initial denaturation step at 95°C for 10 min, followed by 35 cycles. Cycling conditions were as follows: denaturation, 95°C for 30 s; annealing 59°C for 90 s; extension 72°C for 1 min. After the last cycle, a final extension step at 72°C for 10 min was added. Ten microliters of PCR product were analyzed by gel electrophoresis (1.5% gel agarose). Gels were stained with ethidium bromide at 0.5 µl/ml and viewed by UV transillumination. A 100-bp DNA ladder (Amersham Biosciences, France) was used as a size marker. Negative controls were added in each run. Positive PCR controls consisted of *C. jejuni* subsp. *jejuni* ATCC 49943 and *C. coli* ATCC 49941.

Statistical analysis

Data were entered and analyzed with SPSS, version 10 (SPSS Inc, Chicago, USA). The χ^2 test was used for statistical analysis of the significant difference of contamination rates according to the preservation pattern. An α of 0.05 was used for statistical significance.

■ RESULTS

Salmonella was isolated from 96 (32%) of the 300 samples processed, whereas *Campylobacter* was isolated from 168 (56%). Both genera were found together in 54 (18%) samples while 117 samples (39%) were negative for these two bacteria. Eight different *Salmonella* serotypes were isolated from chicken carcasses (Table I). The most prevalent *Salmonella* serovars were *Salmonella* Hadar and *Salmonella* Brancaster. Only two species of *Campylobacter* were recovered from the carcasses; *C. jejuni* was more frequently isolated (59%) than *C. coli* (27%). Both species together were recovered from 14% of the samples.

■ DISCUSSION

Salmonella

Compared to the present study, many authors found a higher prevalence of *Salmonella* in other developing countries: 51.2% in Argentina , 68.2% in Ethiopia , and 72% in Thailand. Conversely, *Salmonella* spp. was detected in only 25.9% of raw broilers in Korea. In developed countries, the levels of *Salmonella* contamination in chicken ranged from 15 to 70% and the average value was about 35% : 16% in Ireland , 22% in the USA , 36.5% in Belgium and 55% in Spain.

Even if the serotypes isolated vary geographically, *Salmonella* Hadar has been frequently isolated from chickens throughout the world. Dominguez et al. in Spain, Jorgensen et al. in the United Kingdom, and Roy et al. in the USA showed that *Salmonella* Hadar was one of the most prevalent serovars in chicken products. However, *Salmonella* Brancaster was not isolated from poultry in

Table I

Salmonella serotypes isolated from chicken meat

***Salmonella* serotypes**	**Isolates**	**%**
Hadar	40	41.6
Brancaster	20	20.8
Agona	8	8.3
Kentucky	8	8.3
Enteritidis	7	7.3
Bredeney	6	6.3
Albany	4	4.2
Wernigerode	3	3.2

any of these studies; only Beli et al. in Albania found this serovar in chicken meat samples. *Salmonella enteritidis* was recovered from only seven samples.

Campylobacter

The present data showed poultry to be prominent reservoirs of *Campylobacter*. In developed countries, several studies have also reported a high proportion of chickens to be contaminated with *Campylobacter* spp.: 46% in Germany , 46% in Japan and from 73 to 100% in the USA. Although little information is available from developing countries, the present results are consistent with those from Kenya and China where thermophilic *Campylobacter* spp. have been isolated from 77 and 76% of chicken samples, respectively. *C. jejuni* was more frequently isolated in the present study. This is in agreement with findings reported by Refregier-Petton et al.. *C. jejuni* is predominantly associated with poultry, while *C. coli* is predominantly found in swine.

Preservation pattern

The contamination rates for *Campylobacter* were significantly different with the preservation pattern ($p < 0.01$): 76% of fresh products were contaminated with *Campylobacter*, whereas only 53% of refrigerated products and 28% of frozen products were contaminated. No difference was noted for *Salmonella* contamination. These results are in agreement with those from Chan et al. , who showed that viability of *Campylobacter* strains was reduced markedly by freezing. These authors also found an ability of the *Campylobacter* isolates to remain viable at 4°C.

Even if the contamination with *Salmonella* and *Campylobacter* generally occurred at the farm , the high contamination in retail chicken meat observed in this study could be explained because of the slaughtering process. Slaughtering usually takes place in traditional butcheries because no modern abattoir is available for poultry. The slaughtering process in these abattoirs is manual and rudimentary, and hygienic conditions are frequently poor. None has automatic functioning. Some people practice slaughtering either inside a specific room or outdoors. Sometimes, only one person does all the work. These conditions increase cross contamination through birds, equipment, and hands of processing-line workers.

The present results showed that chicken carcasses from retail shops proved to be reservoirs of *Salmonella* and *Campylobacter*. Consequently, implementation of good cooking techniques and good kitchen and personal hygiene during preparation are necessary. Moreover there is a strong need to train and educate food handlers in microbial risks associated with poultry meat and how to control them.

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Résumé

Cardinale E., Perrier Gros-Claude J.D., Tall F., Cissé M., Guèye E.F., Salvat G. Prévalence de *Salmonella* et *Campylobacter* dans les carcasses de poulet vendues au détail au Sénégal

Entre janvier 2001 et octobre 2002, 300 carcasses de poulets achetées chez des détaillants à Dakar ont été examinées afin de déterminer la prévalence de *Salmonella* et de *Campylobacter* sur ce type d'aliment. Parmi les carcasses, 146 étaient des produits frais, 58 des produits réfrigérés et 96 des produits congelés. *Salmonella* a été isolée dans 96 (32 p. 100) carcasses. *Salmonella* Hadar (41,6 p. 100) et *Salmonella* Brancaster (20,8 p. 100) ont représenté les sérovars prédominants. *Campylobacter* spp. a été isolé dans 168 (56 p. 100) carcasses. *C. jejuni* a été plus fréquemment identifié (59 p. 100) que *C. coli* (27 p. 100). Les taux de contamination pour *Campylobacter* ont été significativement différents en fonction de la température de conservation des carcasses : cette bactérie a été effectivement isolée dans 76 p. 100 des carcasses conservées à température ambiante, dans 53 p. 100 de celles réfrigérées et dans 28 p. 100 de celles congelées.

Mots-clés : Poulet – *Campylobacter* – *Salmonella* – Congélation – Réfrigération – Sénégal.

Resumen

Cardinale E., Perrier Gros-Claude J.D., Tall F., Cissé M., Guèye E.F., Salvat G. Prevalencia de *Salmonella* y *Campylobacter* en carcasas de pollo a la venta en Senegal

Entre enero 2001 y mayo 2002, se examinaron 300 carcasas de pollo a la venta en tiendas en Dakar, para la prevalencia de *Salmonella* y *Campylobacter*. De éstas, 146 fueron productos frescos, 58 refrigerados y 96 congelados. *Salmonella* se aisló en 96 (32%) de las carcasas examinadas. La sero variedad más importante de *Salmonella* fue *Salmonella* Hadar (41,6%) y *Salmonella* Brancaster (20,8%). *Campylobacter* spp. se aisló en 168 (56%) muestras. *C. jejuni* se aisló más frecuentemente (59%) que *C. coli* (27%). Las tasas de contaminación para *Campylobacter* fueron significativamente diferentes en relación con el tipo de carcasa: 76% frescas, 53% refrigeradas y 28% congeladas.

Palabras clave: Gallo – *Campylobacter* – *Salmonella* – Congelación – Refrigeración – Senegal.