Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods


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Abstract

*Salmonella* and *Campylobacter* continue to be major foodborne pathogens and raw poultry is considered to be an important source of these bacteria. In this study, the prevalence and numbers of *Salmonella* and *Campylobacter* spp. in relation to isolation/sampling methods were determined in 241 whole raw chickens purchased from retail outlets in England during the winters of 1998/1999 (101 chickens) and 1999/2000 (140 chickens). The packaging of the 140 chickens was also examined for the presence of the above pathogens. The prevalence and numbers of enterococci were examined in 21 of the 101 chickens. In total, *Salmonella* and *Campylobacter* spp. were present in 25% and 83% of the chickens, respectively. *Salmonella* was isolated from a sample representing both the inside and outside of the packaging in 19% of the chickens, while the corresponding figure for *Campylobacter* spp. was 56%. Both of these pathogens were isolated from the outside of the packaging in 6% of the chickens. *Salmonella* was more frequently isolated from samples containing chicken skin in comparison with those containing carcass-rinse fluid only. Two chickens (0.8%) were positive for *Salmonella* by direct enumeration methods with contamination levels of log_{10} 3.8 and 4.5 colony forming units (cfu) per carcass, respectively. The most prevalent serotypes were *S*. Hadar, *S*. Enteritidis and *S*. Indiana and two different serotypes were identified in 5/20 salmonella-positive chickens. Resistance to at least one antibiotic was found in 70% of the strains, 46% were multiresistant (resistant to ≥ four drugs) and 52% showed a lowered susceptibility to ciprofloxacin. The likelihood of isolating *Campylobacter* spp. from neck-skin, carcass-rinse or carcass-rinse plus whole skin samples was similar. *Campylobacter* spp. were found in higher levels in carcass-rinse or carcass-rinse plus whole skin samples than in neck-skin. The log_{10} cfu of *Campylobacter* spp. were 2.70–4.99 in 18% of the chickens and 5.00–6.99 in 20%. *Campylobacter* isolates (425) comprised *Campylobacter jejuni* (98%) and *C. coli* (2%) and 98 different serotype/phagetype combinations of these two species were identified. Resistance to at least one antibiotic was found in 73% of the strains and 13% were multiresistant. Thirteen percent of the strains showed lowered susceptibility to ciprofloxacin, while 4.9% were resistant to erythromycin. Vancomycin-resistant enterococci (VRE), able to grow on agar containing 15 mg l^{-1} vancomycin (VRE15), were present in 19 chickens. The log_{10} cfu of VRE15 was 2.90–3.99 in 10 chickens and between 4.00 and 4.99 in two...
chickens. The data presented here contribute to risk assessment and highlight the need to continue to emphasise the safe handling of raw retail poultry. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Isolation methods; Enumeration of Salmonella and Campylobacter; Enterococci; Retail poultry

1. Introduction

The zoonoses which occur most frequently in the industrialised world today are foodborne infections caused by species of Salmonella and Campylobacter. The number of laboratory-confirmed human cases of Campylobacter enteritis in England and Wales increased by 65% from 1991 to 2000 and there were approximately 53,800 cases reported in 2000 (PHLS, 2001a). In 2000, there were about 15,000 laboratory-confirmed cases of Salmonella infection (PHLS, 2001b). While this demonstrated a 13% fall from the 1999 figure and was, in fact, the lowest level for over 10 years, Salmonella still presents a serious risk of foodborne illness. Worldwide, Salmonella and Campylobacter are the most important pathogens associated with poultry products (Bryan and Doyle, 1995). In order to help assess the risks of acquiring infection via undercooked poultry or cross-contamination from chickens, it is important to determine the extent of the contamination of raw poultry with these pathogens.

A national survey conducted in England in 1993/1994 by the Public Health Laboratory Service (PHLS) suggested that Salmonella contamination on poultry carcasses was decreasing and it is important to ascertain such trends in order to verify that intervention strategies employed by the poultry industry are effective (Anon., 1996). National surveys in England and Wales have not included examination for Campylobacter in retail poultry, but a high proportion of raw chicken portions was found to be Campylobacter-positive (Kramer et al., 2000) and data from other countries also show that the prevalence of Campylobacter in poultry can be high (Uyttendaele et al., 1999). Current data on the numbers of Campylobacter and Salmonella spp. on retail chickens are scarce, but a recent study examining 45 chickens for sale on the Dutch market showed that the levels of Campylobacter cells were considerably higher than the levels of Salmonella cells (Dufrenne et al., 2001). Older data suggest that levels of Campylobacter spp. can be very high (>10⁷ per carcass), but the levels on raw chickens for sale in the UK is currently not known (Hood et al., 1988; Pearson et al., 1993).

The method of analysis employed can have a considerable effect on isolation of Salmonella and Campylobacter spp. (Scotter et al., 1993; Anon., 1996; Uyttendaele and Debevere, 1996). Sample and media type and incubation conditions all affect the likelihood of detecting these pathogens. Samples containing neck-skin or other carcass parts have been used in both surveys and for routine product control (Aho, 1992; Anon., 1996). The current method for the detection of Campylobacter approved by the Food Safety and Inspection Service of the US Department of Agriculture, however, can use a whole-bird rinse (Hunt et al., 1998). There is reasonable consensus on method performance for Salmonella isolation techniques and the ISO-recommended method was used in this study (Anon., 1998a). In contrast, there is little consensus on methods for isolating Campylobacter, and a number of different isolation methods are in use worldwide (Corry et al., 1995). The US Food and Drug Administration and ISO methods specify enrichment under microaerobic conditions with various temperature regimes and enrichment broths (Hunt et al., 1998). In a UK-wide survey of raw milk (1674 samples), where the ISO-recommended Park and Sanders method was used, Campylobacter spp. were not isolated from any samples (JFSSG, 1998; Anon., 1998b). A method endorsed by the PHLS has also been used to isolate Campylobacter spp. from raw UK milk, and using this method, 19 of 1097 samples tested were positive (de Louvois and Rampling, 1998). The Exeter selective enrichment broth, on which the PHLS method is based, has been shown to result in improved isolation of Campylobacter from chicken samples in comparison with the Park and Sanders method (Humphrey, 1995).

In addition, because the PHLS method uses a simple temperature regime and enrichment broths are not incubated in a microaerobic atmosphere, we
chose to base the isolation of Campylobacter spp. on this method for the present study. Contaminated poultry meat can harbour vancomycin-resistant enterococci (VRE) (Ike et al., 1999) but data on the numbers of VRE in chickens are scarce. There is also little consensus on how to enumerate/isolate VRE from chickens (Nelson, 1998) and in the present study, a method was examined. Although enterococci are not considered a cause of classic foodborne gastrointestinal illness, resistances present in these bacteria may spread through the food chain and could therefore be considered as an indirect zoonotic problem (Butaye et al., 2000).

The principal aim of the study was to evaluate methods for isolation and enumeration of Salmonella and Campylobacter spp. in raw retail chicken. In Part A of the study, three sample types (neck-skin, carcass-rinse or carcass-rinse with the remaining skin of the chicken) were investigated in relation to the presence of Salmonella and Campylobacter spp. in 101 chickens. The presence of enterococci, in particular, vancomycin-resistant strains, was also determined in 21 chickens. In Part B (140 chickens), isolation and enumeration of Salmonella and Campylobacter spp. from neck-skin samples was compared to that from carcass-rinse plus neck-skin samples. The resulting data on the prevalence and numbers of Salmonella and Campylobacter spp. are discussed in relation to estimating exposure in health risk assessment associated with contamination from chickens.

2. Materials and methods

2.1. Sample collection

For Part A of this study, 75 chilled and 26 raw frozen chickens were purchased from a variety of retail outlets located around Exeter and Preston during November 1998 to January 1999. Giblets were removed, if present, and sample analysis performed (Fig. 1a). For Part B, 70 matched pairs of chilled chickens were purchased from outlets in the Exeter area during November 1999 to February 2000. Pairs of chickens were matched on the basis of size, weight, type and pack-code to compare isolation from neck-skin with that from carcass-rinse with neck-skin samples (Fig. 1b). The outside of the chicken packs and subsequently the entire packaging were also examined (Fig. 1b). The retail outlets included 15 national supermarkets and 3 local butchers.

2.2. Sample preparation

2.2.1. Part A

Two neck-skin samples (weighing 25 and 26 g, respectively) were removed from each chicken, using a sterile scalpel, and placed in individual stomacher bags. The larger neck-skin sample was stomached, using a ‘Lab-Blender 400’ stomacher, for 60 s in 234 ml Buffered Peptone Water (BPW, Oxoid CM509, Oxoid, Basingstoke, Hampshire, UK), then 10 ml was removed for enumeration while the remaining liquid was poured into a sterile honey jar for Salmonella enrichment. The carcass-rinse was prepared by rinsing the chicken, after removal of the neck-skin, in BPW (310 ml) for 90 s in a stomacher bag ensuring contact of the medium with all chicken surfaces by shaking and tilting the bag, repeatedly. Carcass-rinse samples (25 ml) were then removed into separate honey jars for enrichment of Salmonella and Campylobacter spp. After rinsing, the chicken was skinned aseptically and the skin (~ 120 g) was placed in the remaining rinse and stomached for 120 s. Twenty-five-milliliter samples of this homogenate were then removed into separate honey jars for enrichment of Salmonella and Campylobacter spp. The packaging was then removed from the chicken and placed in a stomacher bag; BPW was added to make a 10-fold dilution. Aliquots of 5 ml, for enumeration of Salmonella and Campylobacter spp., and 25 ml (into a honey jar) for enrichment of Campylobacter spp. were removed and the remaining liquid was poured into another honey jar for enrichment of Salmonella spp.
A neck-skin sample (25 g) was removed as described above from one chicken of the pair and placed into a stomacher bag. BPW (225 ml) was added and this was stomached for 120 s. A 5-ml aliquot was removed for enumeration of *Salmonella* and *Campylobacter* spp. Another aliquot (25 ml) was removed into a honey jar for enrichment of *Campylobacter* spp., while the remaining liquid was used for enrichment of *Salmonella*.

A neck-skin sample (25 g) was also removed from the other chicken of a pair and placed into a stomacher bag. The remainder of the chicken was placed into a large stomacher bag and BPW (300 ml) was added. The stomacher bag was tilted 20 times to ensure that the BPW came into contact with all the chicken surfaces. The rinse was then poured into the stomacher bag containing the neck-skin and stomached for 120 s. An aliquot (5 ml) was removed for enumeration of *Salmonella* and *Campylobacter* spp. Another aliquot (25 ml) was removed into a sterile honey jar for enrichment of *Campylobacter* spp., while the remaining liquid was used for enrichment of *Salmonella*.

2.3. Examination of samples for *Salmonella* spp.

### 2.3.1. Enumeration

This was performed by spread-plating 0.5 ml from the samples (neat sample and dilutions thereof in BPW) on Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB; Oxoid CM783) and Xylose Lysine Desoxycholate Agar (XLD; Oxoid CM469), both with added cefsulodin (10 mg l\(^{-1}\)) and novobio-
cin (5 mg l⁻¹). The plates were incubated at 37 °C for 18–24 h.

2.3.2. Enrichment

The enrichment method was based on ISO 6579 (Anon., 1998a). In Part A of the study, BPW (225 ml) was added to the honey jars containing the carcass-rinse and carcass-rinse plus whole skin samples. In Part B, the BPW containing the packaging swabs and packaging material was poured into separate sterile honey jars. Samples (110 ml) of neck-skin and carcass-rinse plus neck-skin were added to three separate honey jars. Novobiocin (5 mg l⁻¹) and cefsulodin (10 mg l⁻¹) were added to one of the jars containing a carcass-rinse plus neck-skin sample.

All samples were then subjected to pre-enrichment for 16–20 h at 37 °C. Volumes (0.1 ml) of the pre-
enriched cultures were added to 10 ml Rappaport–Vassiliadis Soya Peptone Broth (RVS; Oxoid CM866) and these were incubated for selective enrichment at 41.5 °C. Ten milliliters was also added to 100 ml of Selenite Cystine Broth (Oxoid CM699) with added Sodium Biselenite (SCB; Oxoid L121), and these were then incubated for selective enrichment at 37 °C. After 24 and 48 h, a 10-μl loop was streaked from the selective enrichment broths onto modified Brilliant Green Agar (mBGA; Oxoid CM329) and XLD. These plates were incubated for 24 h at 37 °C.

2.3.3. Confirmation and subtyping

Presumptive colonies (three to five from each sample) were confirmed using MacConkey Agar (Oxoid CM7), Urea Broth (Oxoid CM71 with SR20 supplement) and Triple Sugar Iron Agar (Oxoid CM277). All biochemical tests were incubated at 37 °C for 24 h. Isolates showing typical Salmonella biochemical reactions were tested with polyvalent antisera for O and H antigen. Confirmed isolates, obtained via enrichment, were stored and, where possible, at least one isolate per sample was further characterised by serotyping, phage typing and tests for resistance to ampicillin (A), chloramphenicol (C), reduced sensitivity to ciprofloxacin (Cp), streptomycin (S), sulphonamide (Su), spectinomycin (Sp), tetracycline (T), trimethoprim (Tm), nalidixic acid (Nx), gentamicin (G), kanamycin (K) and furazolidone (Fu) (Kauffmann, 1964; Anderson, 1964; Threlfall et al., 1999; Frost, 1994) at the Laboratory for Enteric Pathogens, Central Public Health Laboratory (CPHL).

2.4. Examination of samples for Campylobacter spp.

2.4.1. Enumeration

Samples of 0.5 ml (neat sample and/or dilutions in BPW) were spread-plated onto modified charcoal cefaperazone desoxycholate agar (CCDA, Oxoid CM739 with SR155 supplement). All plates were incubated in a microaerobic atmosphere at 37 °C for 48 h using gas jars (Bolton et al., 1992). In Part B, Campylobacter spp. were also enumerated in successive carcass-rinse samples of 13 additional chickens in order to estimate the proportion of cells recovered from one rinse. These carcasses were rinsed with 300 ml of BPW for 2 min. This procedure was repeated to give a total of three rinses for each chicken. Samples were spread-plated onto CCDA agar plates and these were incubated as previously described.

2.4.2. Enrichment

Isolation of Campylobacter spp. was based on the method endorsed by the PHLS (Anon., 1998c) and used a modified Exeter Broth (EB) (Humphrey et al., 1995) containing Nutrient broth (25 g l⁻¹; Mast DM180, Mast Diagnostics, Bootle, Merseyside, UK), Campylobacter Growth Supplement (sodium metabisulphate, sodium pyruvate and ferrous sulphate, all at 250 mg l⁻¹; Mast SV61), Campylobacter Selective Supplement (trimethoprim 10 mg l⁻¹, rifampicin 5 mg l⁻¹, polymyxin B 2500 IU l⁻¹, cefaperazone 15 mg l⁻¹, amphotericin B 2 mg l⁻¹; Mast SV59) and lysed defibrinated horse blood (10 ml l⁻¹; E&O Laboratories, Bonnybridge, Scotland) for enrichment. In Part A, the neck-skin sample was stomached for 1 min in 225 ml EB and then poured into a honey jar. Modified EB (225 ml) was also added to the carcass-rinse and carcass-rinse plus whole skin samples and to each of the outer packaging, entire packaging, neck-skin and carcass-rinse plus neck-skin samples from Part B. All samples were then subjected to enrichment culture for 48 h at 37 °C in a normal atmosphere. Then, 10 μl of each broth was streaked onto CCDA and the plates were incubated at 37 °C for 48 h in a microaerobic atmosphere.

2.4.3. Confirmation and subtyping

Presumptive colonies (three to five for each sample) were confirmed by testing for growth on blood agar (BA, Columbia agar base [Oxoid, CM331] with 5% defibrinated horse blood) in aerobic and microaerobic atmospheres at 37 °C for 48 h, by oxidase activity and by examination of cell morphology using Gram-staining and microscopy. Confirmed isolates, obtained via enrichment, were stored, and, where possible, at least one isolate per sample was sent to the Campylobacter Reference Unit (CRU) on Amies charcoal transport swabs (Technical Service Consultants, Haywood, Lancashire) for speciation, serotyping (using the Laboratory of Enteric Pathogens heat-stable (HS) antigen serotyping scheme), phage typing and tests for resistance to A, C, Cp, T, Nx, G, K, erythromycin and neomycin (Frost et al., 1998, 1999; Salama et al., 1990; Thwaites and Frost, 1999).
2.5. Examination of samples for enterococci

Enterococci were enumerated in carcass-rinse plus whole skin samples from 21 chickens. Enumeration was carried out by plating volumes (0.5 ml; neat sample and dilutions thereof) onto BBL Enterococcosel agar (Becton Dickinson 4312205; Oxford, UK) with added clindamycin (2.25 g l\(^{-1}\)) containing either none (EA), 6 (EA6) or 15 (EA15) mg vancomycin l\(^{-1}\) to differentiate between vancomycin-susceptible (VSE) and resistant enterococci (VRE) (Edberg et al., 1994). These plates were incubated at 37 °C in a 6% CO\(_2\) incubator for 24 h. The EA plates were assessed for their ability to grow colonies of \(\text{vanA}\) and \(\text{vanB}\) type strains, as controls. Both \(\text{vanA}\) and \(\text{vanB}\) cultures grew on EA6 plates, but only the \(\text{vanA}\) strain was able to grow on EA15 plates.

Enrichment of enterococci was performed by adding BBL Enterococcosel broth (Becton Dickinson 12207; 225 ml) to the chicken samples. These were incubated for 24 h at 37 °C and then streaked onto EA, EA6 and EA15. The plates were incubated at 37 °C for 24 h in a 6% CO\(_2\) incubator. Confirmation of presumptive positive enterococci colonies (three to five black colored colonies for each sample) was performed by subculturing onto BA and Bile Aesculin (Oxoid CM888) slopes. These were incubated for 24 h at 37 °C. Further confirmatory tests included Gram-staining, catalase test and latex agglutination test for group D streptococci.

2.6. Statistical analysis

The agreement between isolation methods from different sample types was assessed using the chi-square test.

3. Results

3.1. Isolation of Salmonella in relation to sample type

Salmonella were isolated from 31% of the chickens in Part A and from 21% in Part B (Table 1). In Part A, significantly fewer Salmonella were isolated from carcass-rinse samples than from either neck-skin or carcass-rinse plus whole skin samples \((P<0.001\) and \(P<0.05\), respectively; Table 2). With six chickens, only the neck-skin sample was Salmonella-positive, with four others, Salmonella were only isolated from the carcass-rinse plus whole skin samples, while no chickens were positive only by examination of the carcass-rinse samples. There was no significant difference in Salmonella isolation rates from either neck-skin only or carcass-rinse plus neck-skin samples in Part B (Table 2). Salmonella were isolated from the entire packaging in 25/140 chickens, including eight where the corresponding neck-skin or carcass-rinse plus neck-skin sample was negative, and from the outer packaging of 9/140 chickens (Table 2). The study included 27 chickens which were of non-UK origin and 14 (52%) of these were Salmonella-positive.

3.2. Isolation of Salmonella in relation to enrichment method

Salmonella were isolated from 17/70 samples tested when the pre-enrichment was performed in the presence of novobiocin and cefsulodin, and from 14 samples when these antibiotics were absent. Five samples were positive only when these antibiotics were present and only two when the antibiotics were absent. Salmonella was isolated from significantly more samples by enrichment in RVS compared to that in SCB \((P<0.001, \chi^2=18; \) Table 3). Seven samples were only positive following enrichment in SCB, but this compared to 45, which were only positive, when RVS was used. Salmonella was usually isolated after both 24 and 48 h of selective enrichment irrespective of the medium used, but 6/130 samples...
were only positive after 48 h and 5/130 only after 24 h.

3.3. Numbers of Salmonella

Two of the one hundred and one chickens examined in Part A were positive for Salmonella by direct enumeration (none of the chickens from Part B had levels of Salmonella above the detection limit). The log_{10} cfu of Salmonella in the neck-skin sample of one chicken was 3.8, and the other had 4.3 and 4.5 in the carcass-rinse and carcass-rinse plus whole skin samples, respectively. The detection limit of the direct enumeration for neck-skin, carcass-rinse and carcass-rinse plus whole skin samples was 500, 700 and 800 cells, respectively in Part A. In Part B, it was 250 cfu per chicken for the packaging and neck-skin samples and 300 cfu per chicken for the carcass-rinse plus neck-skin samples.

3.4. Salmonella serovars, phage types and antibiotic resistance

Fifteen different Salmonella serotypes were identified among the 119 strains typed including S. Hadar (28% of chickens), S. Enteritidis and S. Indiana (16% each), S. Thomson and S. Virchow (6.7% each), S. Heidelberg (4.9%), S. Agona, S. Anatum, S. Breedeny and S. Typhimurium (3.3% each) and S. Infantis, S. Kentucky, S. Livingstone, S. Newport and S. Worthington (1.6% each). Two different serotypes were identified in 5 of 20 chickens from which at least two strains were typed. The S. Enteritidis isolates were all PT 4, while the S. Typhimurium strains were DT 104. In Part A (47 strains), antibiotic resistance to at least one of the drugs tested was found in 85% of the strains, 34% were resistant to ≥ four antibiotics (multiresistant), and a lowered susceptibility to ciprofloxacin was observed in 53% of the strains. In Part B (72 strains), antibiotic resistance to at least one of the drugs tested was found in 62% of strains, 53% were multiresistant and a lowered susceptibility to ciprofloxacin was observed in 50% of the strains. There was no significant difference in the proportion of ciprofloxacin-resistant strains between those isolated from either UK or non-UK chickens.

3.5. Isolation of Campylobacter spp. in relation to sample type

Campylobacter spp. were isolated from a higher proportion of the chickens examined in Part A of this study than in Part B (Table 1). The likelihood of isolating Campylobacter spp. from samples of neckskin (25 g), carcass-rinse (25 ml) or carcass-rinse plus whole skin (~17 ml rinse fluid + 8 g skin) was similar and at least two of three samples types were positive

Table 2
Isolation of Salmonella and Campylobacter spp. from raw chickens in relation to sample type

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample type</th>
<th>No. of positive samples/no. examined (% positive)</th>
<th>Salmonella spp.</th>
<th>Campylobacter spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A</td>
<td>neck-skin</td>
<td>26/101 (26) 85/101 (84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>carcass-rinse</td>
<td>13/101 (13) 87/101 (86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>carcass-rinse plus whole skin</td>
<td>23/101 (23) 91/101 (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part B</td>
<td>neck-skin</td>
<td>11/70 (16) 51/70 (73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>carcass-rinse</td>
<td>14/70a (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>carcass-rinse plus neck-skin</td>
<td>17/70b (24) 55/70 (79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>entire packaging</td>
<td>25/140 (19) 79/140 (56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>outer packaging</td>
<td>9/140 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Pre-enriched without novobiocin and cefsulodin.
b Pre-enriched with novobiocin and cefsulodin.
c Pre-enriched either with or without novobiocin and cefsulodin.

Table 3
Isolation of Salmonella from raw chickens in relation to selective enrichment broth

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of positive samples(^a) (% of no. of positive in both media)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rappaport–Vassiliadis soy peptone broth</td>
</tr>
<tr>
<td>Neck-skin</td>
<td>34 (92)</td>
</tr>
<tr>
<td>Carcass-rinse</td>
<td>13 (100)</td>
</tr>
<tr>
<td>Carcass-rinse plus whole skin</td>
<td>22 (96)</td>
</tr>
<tr>
<td>Carcass-rinse plus neck-skin</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Entire packaging</td>
<td>24 (96)</td>
</tr>
<tr>
<td>Outside packaging</td>
<td>7 (78)</td>
</tr>
<tr>
<td>Total</td>
<td>130 (96)</td>
</tr>
</tbody>
</table>

\(^a\) All samples from Part A and Part B.
in 92/101 chickens (Table 2). Although more chickens were positive if examined by a neck-skin plus carcass-rinse sample (containing ~ 22.5 ml rinse fluid + 2.5 g neck-skin) than by a neck-skin sample (containing ~ 2.5 g neck-skin), this was not significant (Table 2, Part B). *Campylobacter* spp. were only slightly less prevalent in frozen than chilled chickens with 85% neck-skin, 81% carcass-rinse and 85% carcass-rinse plus whole skin being positive as compared with 89%, 91% and 95%, respectively for chilled chickens. *Campylobacter* spp. were also isolated from 56% of entire packaging samples which represented the contamination present on both the inside and outside of the packaging, and from 6% of the outer packaging (Table 2).

### 3.6. Numbers of *Campylobacter* spp.

*Campylobacter* spp. were detected by direct plating in 40% of chickens from Part A and 36% from Part B. The likelihood of detecting *Campylobacter* spp. by direct plating was similar for most samples types, except for entire packaging samples where detection by direct plating occurred in a much lower proportion of the samples (Fig. 2). In Part A, higher levels of *Campylobacter* spp. were found in carcass-rinse and carcass-rinse plus whole skin samples than in neck-skin samples and the \( \log_{10} \) geometric means were 4.9 (SD = 1.0), 5.1 (SD = 1.1) and 4.1 (SD = 0.7), respectively. The \( \log_{10} \) geometric mean can be calculated as the average of the \( \log_{10} \) counts. The means included only those chickens where a direct count was obtained from all three samples. Occasionally, direct counts were only obtained from one of the three sample types, but such counts were usually just above the detection limit. In Part A, one chicken had > 9 \( \log_{10} \) cfu of *Campylobacter* spp. in the carcass-rinse plus whole skin and carcass-rinse samples. It was only possible to enumerate *Campylobacter* spp. in 4 out of 26 frozen chickens by the direct plating method (range: 2.8–4.4 \( \log_{10} \) cfu per chicken). The limits of detection for *Campylobacter* spp. by direct enumeration was 500 cfu for the entire packaging and neck-skin samples and 600 cfu for the carcass-rinse plus neck-skin samples. The number of *Campylobacter* cells removed from a chicken carcass in three con-

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**Fig. 2.** Distribution of the numbers of *Campylobacter* spp. in neck-skin (open bars), carcass-rinse (striped bars), carcass-rinse with whole skin (hatched bars), carcass-rinse with neck-skin (dotted bars) and entire packaging (black bars) samples.
secutive rinses was compared to obtain an estimate of the proportion of cells remaining on the carcass after one rinse. The log10 geometric mean of *Campylobacter* cells recovered from 13 carcasses in the first rinse was 4.8 (SD = 0.4), while the second and third rinse contained 4.5 (SD = 0.4) and 4.2 (SD = 0.5).

### 3.7. Serotypes, phage types and antibiotic resistance of *Campylobacter* isolates

*Campylobacter* isolates (425) were speciated, sero- and phage-typed and tested for antimicrobial resistance. Forty-one serotypes of two species (98% *Campylobacter jejuni* and 2% *C. coli*) were identified. A wide range of phagetypes was identified within each of the *C. jejuni* serotypes and the combined serotype and phagetype data identified 98 sero/phagetypes. Seventeen percent were untypable by serotyping, 8% by phagetyping, but only 1.6% did not type using either method. More than one strain was typed from 74% of the *Campylobacter*-positive chickens and contamination with more than one distinct strain, either by species or serotype or phagetype or sero/phagetype was confirmed in 68% of positive samples (Table 4). Sixty-six percent of the *Campylobacter* isolates, from Part A and 72%, from Part B showed resistance to at least one of the antibiotics tested. Thirteen percent of the *C. jejuni* and 16% of *C. coli* strains from Part A and 7% from Part B were multi-

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of chickens (% of positive) with</th>
<th>&gt;1 Strain typed</th>
<th>Different sero- and phage types</th>
<th>Either different sero- or phage type</th>
<th>Two species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A</td>
<td>84 (90)</td>
<td>23 (25)</td>
<td>20 (22)</td>
<td>11 (12)</td>
<td></td>
</tr>
<tr>
<td>Part B</td>
<td>64 (59)</td>
<td>19 (18)</td>
<td>22 (21)</td>
<td>5 (5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>148 (74)</td>
<td>42 (21)</td>
<td>42 (21)</td>
<td>16 (8)</td>
<td></td>
</tr>
</tbody>
</table>

* Strains isolated from neck-skin, carcass-rinse and carcass-rinse plus whole skin samples.

* Strains from each chicken from neck-skin and entire packaging samples or carcass-rinse plus neck-skin and entire packaging samples.

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**Fig. 3.** Distribution of the numbers of enterococci able to grow on agar with no vancomycin (VSE), on agar containing 6 mg l⁻¹ vancomycin (VRE6) and on agar containing 15 mg l⁻¹ vancomycin (VRE15) in carcass-rinse with whole skin samples from 21 chickens.
resistant. Resistance to erythromycin was common in *C. coli* (56%), but rare in *C. jejuni* (1.8%). Twelve percent of strains from Part A and 13% from Part B were resistant to ciprofloxacin and the proportion of resistant isolates of non-UK origin was not significantly different to that of strains isolated from chickens produced in the UK. Different *Campylobacter* species, serotypes and phage types were equally likely to be recovered from the different sample types.

### 3.8. Isolation and enumeration of enterococci

Vancomycin-resistant enterococci (able to grow on EA15) were present in 19 chickens and a majority (12/21) had > 2.90 VRE per chicken (Fig. 3). The geometric average of VRE in chickens was log_{10} 3.4 and 3.1 on EA6 or EA15, respectively. The means included carcass-rinse plus whole skin samples which were positive by enrichment but not direct plating, and the level of enterococci in those samples was arbitrarily set to half the detection limit of the direct plating method.

### 4. Discussion

In this study, the presence of *Salmonella* and *Campylobacter* spp. in different raw chicken samples was examined by enrichment and by direct plating to enable enumeration. The isolation rate of *Salmonella* from carcass-rinse samples was significantly lower than from those containing skin. This could be related to cells which are firmly attached to the chicken skin (Lillard, 1986, 1988), and which are not easily dislodged by rinsing, but other factors including differences in background flora may also play a role. The likelihood of detecting *Campylobacter* spp. in a chicken appeared to be less influenced by sample type, but examination of samples containing carcass-rinse fluid and neck-skin detected a higher *Campylobacter* count in more chickens than examination of the neck-skin sample alone. Taken together, the studies indicate that examination of a carcass-rinse plus neck-skin sample provides a representative and convenient sample for the detection and enumeration of *Salmonella* and *Campylobacter* from whole raw chicken. The use of RVS broth resulted in more frequent isolation of *Salmonella* than the use of SCB, but the use of both broths was sometimes beneficial. For example, the prevalence of *Salmonella* from neck-skin samples with RVS enrichment was 24%, as compared to 26% when both RVS and SCB was used. The present study confirms that the PHLS method is suitable for the isolation of *Campylobacter* from chickens. Improvement of the efficacy of the broth has been suggested by delayed addition of some of the antibiotics (Humphrey et al., 1996). This could potentially improve recovery of sub-lethally damaged cells and may be particularly relevant for frozen samples.

Direct comparison of the prevalence of these pathogens reported by different authors is hampered due to differences in time period, sampling technique and isolation methods (Uyttendaele et al., 1999). The prevalence of *Salmonella* (21%) found in the chilled chickens in this study was, however, lower than that (28%) found in a survey conducted by the PHLS in 1994 in chilled UK-produced chickens by analysis of neck-skin (Anon., 1996), but not significantly so ($\chi^2 = 2.87$, $p = 0.09$). This could suggest that contamination levels in retail chicken have decreased, but the data sets may not be directly comparable due to the different sampling techniques. Another recent study examined 133 chicken carcasses at retail sale in Belgium and reported a 34% *Salmonella* prevalence (Uyttendaele et al., 1999). Only two chickens contained levels of *Salmonella* in sufficient numbers to allow their detection by direct plating onto *Salmonella*-selective agars, suggesting that the majority of the positive chickens contained relatively low levels of *Salmonella*. Although the direct enumeration method is likely to underestimate the actual numbers of *Salmonella*, these data suggest that the number of cells present on carcasses is low in agreement with a recent study (Dufrenne et al., 2001). The proportion of *Salmonella*-positive chickens contaminated with *S. Enteritidis* was lower in this study (16%) than the 45–60% reported for chickens examined in 1994 (Anon., 1996), and could relate to the widespread introduction of a *Salmonella Enteritidis* vaccine in England and Wales in 1997 (Anon., 2000).

The prevalence of *Campylobacter* spp. in whole chickens found in this study (83%) was very similar to that reported by Kramer et al. (2000) and Bolton et al. (1999) who isolated *Campylobacter* spp. from 83% (165/198) of chicken portions and 86% (290/336) of poultry products at retail sale on the English market,
respectively. A recent Dutch study also reported >80% of 45 raw chickens to be positive for Campylobacter spp. while a lower percentage (29%) was reported for raw poultry products for sale on the Belgian market (Uyttendaele et al., 1999; Dufrenne et al., 2001). A study from 1997 showed 229 of 330 raw chicken carcasses for sale in North Carolina, USA to be positive for C. jejuni (Willis and Murray, 1997). Although relatively high numbers of Campylobacter spp. (≥ log_{10} 5.0) were found on ~20% of chickens in this study, >50% of 28 chickens examined in 1986 had > log_{10} 6.0 Campylobacter spp. per chicken (Pearson et al., 1993). A recent study of chickens for sale on the Dutch market found that 18% of 45 chickens had > 5500 Campylobacter cells per carcass, while in this study 36% of chickens had > 5500 Campylobacter cells (Dufrenne et al., 2001). The risk of cross-contamination arising from chickens may be proportional to the number of cells present and could explain why one study has shown that Campylobacter spp. were more frequently associated with cross-contamination events than Salmonella during the handling of raw chickens (de Boer and Hahné, 1990). In agreement with others, we also found that the levels of Campylobacter spp. in frozen chickens were lower than in chilled chickens (Dufrenne et al., 2001) and this may relate to the freeze-damaged cells encountered on frozen carcasses. The 10 most common C. jejuni serotypes accounting for 51% of the isolates reported by Kramer et al. (2000) also accounted for half of the chicken strains identified in the present study. These authors also found that distribution of C. jejuni subtypes isolated from humans was similar to that observed in chicken isolates when strains from the same time period and geographical area were examined. Data for the distribution of subtypes from human isolates, from the same geographical area and time period the Campylobacter strains in this study were isolated, are unfortunately, not available.

The presence of Salmonella and Campylobacter spp. on the outer packaging raises concern as consumers would not expect products to be contaminated on the outside and would consequently do nothing to avoid cross-contamination. A similar prevalence of contamination of the outer packaging of chicken portions with Campylobacter spp. (5.4%) has been reported recently (Bolton et al., 1999). The number of organisms present is probably low and the risk relating to cross-contamination may therefore be small, but the studies do highlight a largely unrecognised risk-factor which may have implications for the transmission of Salmonella and Campylobacter spp.

Enterococci able to grow in the presence of 15 mg vancomycin 1^{-1} were present in 19 out of the 21 chickens examined, with numbers of VRE ranging from < log_{10} 2.7 to log_{10} 4.4 per chicken. Studies in other countries similarly have revealed frequent isolation of VRE from poultry (Klare et al., 1995a,b; Bager et al., 1997; Robredo et al., 2000) and the high prevalence of VRE found in this study confirms that raw poultry is a significant reservoir for VRE and therefore a possible source of VRE infections. The VRE strains were isolated in January 1999, while the European Community banned the use of a related antibiotic, avoparcin, for veterinary use in April 1997, suggesting, in agreement with other studies, that a reduction in resistance rates after cessation of drug use may be slow (Austin et al., 1999).

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References

Anon., 1998c. PHLS Methods for Food Products: Detection of


de Louvois, J., Rampling, A., 1998. One fifth of samples of unpasteurised milk are contaminated with bacteria. BMJ. 316, 625–626.

Dufrenne, J., Ritmeester, W., Delfgou-van Asch, E., van Leusden, F., de Louvois, J., 1994. One fifth of samples of unpasteurised milk are contaminated with bacteria. BMJ. 316, 625–626.


