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Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey

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Abstract

Mean counts of Enterobacteriaceae were determined for 30 samples each of organic chicken meat, conventional chicken meat and conventional turkey meat to assess differences in contamination. Two strains from each sample were isolated to obtain a total of 180 strains, which were examined for resistance to ampicillin, chloramphenicol, cephalothin, doxycycline, ciprofloxacin, gentamicin, nitrofurantoin, and sulfisoxazole. The mean counts of Enterobacteriaceae from organic chicken meat were significantly higher than those obtained from conventional chicken ($P < 0.0001$) or conventional turkey ($P < 0.0001$) meat. However, the resistance data obtained showed that isolates from organic chicken meat were less resistant than isolates from conventional chicken meat to ampicillin ($P = 0.0001$), chloramphenicol ($P = 0.0004$), doxycycline ($P = 0.0013$), ciprofloxacin ($P = 0.0034$), gentamicin ($P = 0.0295$) and sulfisoxazole ($P = 0.0442$), and were less resistant than isolates from turkey meat to doxycycline ($P = 0.0014$) and sulfisoxazole ($P = 0.0442$). Multidrug resistant isolates were found in every group tested, but rates of multidrug resistant strains were higher in conventional chicken (63.3%) and turkey (56.7%) than organic chicken (41.7%) meat. The rates obtained for antimicrobial resistance support the theory that although organic chicken meat contains more Enterobacteriaceae contamination, organic farming practices contribute to decreased dissemination of antibiotic resistance.

Keywords: Poultry; Organic; Enterobacteriaceae; Antimicrobial; Resistance

1. Introduction

Organic and other non-conventional meat products are now readily available for retail in developed countries, to satisfy consumers' demand for high-quality products that meet the following requirements: (i) guaranteed animal welfare during production; (ii) absence of chemical agents during animal feeding; (iii) environmental-friendliness, and (iv) better taste than conventional products (Dransfield et al., 2005). However, little is known about the microbiological status of organic animal products and the potential microbiological risks linked to organic meat production. Thus, raising of animals outdoors, use of slow-growing breeds, strict restrictions in the therapeutic use of antimicrobial agents and use of small slaughtering facilities may not guarantee strict microbiological control of animals destined for human consumption (Dransfield et al., 2005; Soonthornchaikul et al., 2006).

Currently, it is well known that several antimicrobial-resistant bacteria isolated from humans originate primarily

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from animals raised for human consumption (Aarestrup, 2000) and that such resistant bacteria may contaminate the meat derived from those animals (Sáenz et al., 2001). Although this contamination declines in the absence of antimicrobial agents (Phillips et al., 2004), the presence of antimicrobial-resistant bacteria may persist in meat even after the withdrawal period (Van den Bogaard, London, Driessen, & Stobberingh, 2001; Wiuff, Lykkesfeldt, Svendsen, & Aarestrup, 2003). Thus, the development of antibiotic resistance among bacterial isolates from animal sources can also represent a potential hazard to consumers *via* food-borne infections caused by antibiotic-resistant bacteria.

The Enterobacteriaceae family is commonly used as an indicator of faecal contamination during food microbiological analyses, and includes important zoonotic bacteria such as *Salmonella* spp., *Yersinia* spp. and *Escherichia coli*. Enterobacteriaceae are the significant causes of serious infection, and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials (Paterson, 2006). This is an important phenomenon that requires vigilance and find measures to control the further spread of resistance by pathogens included in this family.

Recently, antimicrobial resistance has been reported in bacteria isolated from organic dairy products (Sato, Barlett, Kaneene, & Downes, 2004; Sato, Bennedsgaard, Barlett, Erskine, & Kaneene, 2004; Tikofsky, Barlow, Santiesteban, & Schukken, 2003), and in poultry products related to *Salmonella* and *Campylobacter* (Cui, Ge, Zheng, & Jianghong, 2005; Soonthornchaikul et al., 2006). However, little information relative to commensal bacteria isolated from organic poultry meat products is currently available. Consequently, the main goal of the present study was to investigate the prevalence of antimicrobial susceptibility found in Enterobacteriaceae isolates derived from organic chicken meat as compared to conventional chicken and turkey meats. The potential implications of these results in terms of microbiological safety, especially concerning the development and spread of antimicrobial resistance to the food chain, are also discussed.

2. Methods

2.1. Collection of poultry meat samples

A total of 90 fresh pre-packaged skin-on drumstick samples were taken during 2005 from supermarkets and butcher shops: 30 organic-reared chicken samples, 30 conventionally-reared chicken samples, and 30 conventionally-reared turkey samples. All samples were taken on different days and in different supermarkets and butcher shops for the case of conventional poultry. For the case of organic chickens, certified products only were found in five supermarkets, so six organic samples were taken in each supermarket, but all of them on different days. All supermarkets and butcher shops were located in Galicia (North-Western Spain). All samples were processed between three and four days before the expiration date indicated on the label.

2.2. Microbiological analyses

All samples were processed following standard performance ISO Standard 7402 (1993) for plate count of Enterobacteriaceae: Portions of 25 g were obtained from each meat sample, placed in a sterile masticator bag with an appropriate volume (1/9) (w/v) of sterile 0.1% peptone water (Merck, Darmstadt, Germany), and subsequently homogenized for 1 min with a masticator (Aes, Combourg, France). After homogenization, samples were investigated for the presence of Enterobacteriaceae. Thus, 10^{-1}–10^{-4} dilutions of meat extracts were tested on poured plates of Crystal-violet neutral-red bile glucose agar (VRBG), which were prepared as specified by the manufacturer (Merck). Once the agar had solidified, plates were overlaid with 3–4 ml of melted VRBG and incubated at 35–37 °C for 24 h. After incubation, red colonies were identified as Enterobacteriaceae and counted.

Once the bacterial counts were determined, two typical Enterobacteriaceae colonies isolated from each meat sample were picked, transferred onto Columbia agar supplemented with 5% sheep blood (BioMérieux, Marcy l'Etoile, France), and incubated at 35–37 °C for 24 h in order to obtain a total of 180 pure cultures. Such pure cultures were identified by colony and cell morphology, Gram stain, oxidase and catalase activity. Positive strains initially identified as Enterobacteriaceae were identified by API 20 E (BioMérieux) identification tests.

All 180 Enterobacteriaceae isolates were stored at −80 °C in Maintenance Freeze Medium units (Oxoid, Basingstoke, UK) until antimicrobial susceptibility testing.

2.3. Antimicrobial susceptibility testing of bacteria

Antimicrobial susceptibility testing was performed for a total of 180 isolates of Enterobacteriaceae (60 from organic chicken meat, 60 from conventional chicken meat and 60 from conventional turkey meat). Antimicrobial susceptibility testing was carried out on Mueller–Hinton agar plates (Biomerieux) by the agar disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, Formerly NCCLS, 2002). Antimicrobial disks considered were: ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), doxycycline (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), nitrofurantoin (300 μg) and sulfisoxazole (300 μg) (Oxoid). The antibiotic resistance breakpoints considered were the interpretative criteria for Enterobacteriaceae recommended by the CLSI (2002). *E. coli* ATCC 25922 was used as quality control.

Antimicrobials were chosen on the basis of their ability to provide a diverse representation of different antimicrobial agent classes. Enterobacteriaceae isolates were classified as sensitive, intermediate or resistant. Isolates