



Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia* spp. isolated from retail chicken and beef, Tehran, Iran

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ABSTRACT

Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia* spp. isolates from fresh chicken and beef meat obtained at retail outlets in Tehran, Iran, were determined. A total of 379 samples (190 chicken and 189 beef) were collected between April 2006 and April 2007 and analyzed for these foodborne pathogens, and isolates were subjected to antimicrobial resistance testing. *Salmonella*, *Campylobacter* and *Yersinia* were isolated from 124 (33%), 109 (29%) and 60 (16%) of the samples, respectively. *S. Thompson* was the dominant serovar of *Salmonella*, isolated from 75% to 58% of the *Salmonella*-positive chicken and beef samples, respectively, with *S. Hadar* a distant second. Of *Campylobacter* isolates, 71% were resistant to nalidixic acid and 47% to ciprofloxacin, with 37 (40%) multidrug resistant. A high percentage of *Salmonella* isolates were resistant to antibiotics, including 82% nalidixic acid, 69% tetracycline, 63% trimethoprim and 52% streptomycin. Eighty-five (68.5%) isolates were multidrug resistant. The most *Yersinia* spp. were resistant to cephalotin 59 (98%) and ampicillin 31 (52%), with 14 (23%) multidrug resistant. Such baseline information on the prevalence and antimicrobial resistance of foodborne pathogens on raw foods of animal origin is needed to not only determine the extent of the problem but also to serve as a point of reference for monitoring changes that occur over time. Such data are essential for use in developing effective risk management strategies.

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1. Introduction

The microbiological safety of foods is an increasing public health concern worldwide. Many epidemiological studies have implicated foods of animal origin as major vehicles associated with illnesses caused by *Campylobacter*, *Salmonella* and *Yersinia* spp. (Centers for Disease Control, 2008; Cretikos, Telfer, & McNulty, 2008). Contaminated raw or undercooked poultry and red meat are particularly important in transmitting these foodborne pathogens (Vindigni et al., 2007). Antimicrobial treatment of human *Campylobacter* enteritis, salmonellosis and yersiniosis is in most cases not indicated. However, for severe cases, extraintestinal dis-

ease, or for infections of immunocompromised persons, antibiotic therapy may be necessary, but in some cases, therapy may be complicated by antimicrobial resistance of pathogens infecting humans (Engberg, Neimann, Nielsen, Aarestrup, & Fussing, 2004). Antibiotics have been used successfully in poultry for different purposes such as growth promotion, prophylaxis, or therapeutics. However, their use in animal production and human therapy has resulted in increased bacterial resistance to many antibiotics (Castanon, 2007; Mathew, Cissell, & Liamthong, 2007). As a result, chicken and meat can harbor antimicrobial-resistant strains that can be transmitted to humans. There is no published information in Iran regarding the prevalence of *Campylobacter*, *Salmonella* and *Yersinia* isolated in food and their antibiotic susceptibility. The purpose of this study was to determine the prevalence and antimicrobial resistance profiles of these foodborne pathogens isolated from raw chicken and beef in Tehran, Iran.

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2. Materials and methods

2.1. Sample collection

A total of 379 samples, including chicken ($n = 190$) and beef ($n = 189$), were collected at retail outlets in Tehran, Iran, between April 2006 and April 2007. All samples were transported on ice to the National Research Department of Foodborne Diseases (NRDFD) and microbiological analyses were carried out within 2 h after collection.

2.2. Isolation and identification procedure

Meat samples (25 g each) were homogenized in a stomacher (Stomacher 400 Circulator, Seward, UK) in 225 ml of buffered peptone water (Oxoid) for 1 min. It was incubated at 37 °C for 24 h. Then, 1 ml of broth enriched in *Campylobacter* enrichment broth base supplemented with *Campylobacter* selective supplement IV (Himedia, India) and 5% defibrinated sheep blood. After incubation at 42 °C for 24 h in a microaerophilic environment (5% O₂, 10% CO₂, 85% N₂), 0.1 ml was streaked onto *Campylobacter* selective agar base (Himedia, India) containing an antibiotic supplement for the selective isolation of *Campylobacter* species (Himedia, India) and 5% (v/v) defibrinated sheep blood and incubated for 48 h at 42 °C under the same conditions. One presumptive *Campylobacter* colony from each selective agar plate was subcultured, then Gram stained and tested for production of catalase, oxidase and hippurate hydrolysis were done. Hippurate hydrolysis was assessed by rapid hippurate hydrolysis test using ninhydrin.

For *Salmonella* isolation, 25 g of sample was preenriched with 225 ml of buffered peptone water at 37 °C for 24 h. After incubation, 1.0 ml of the preenrichment culture was transferred into 9.0 ml of tetrathionate broth and incubated at 42 °C for 24 h. The enrichment culture was streaked onto XLT4 (Difco) agar plates and incubated for 24 h at 37 °C. Presumptive *Salmonella* colonies were confirmed by using API 20E (bioMérieux 20100). Agglutination tests were done with *Salmonella* polyvalent O and H antisera (Mast Diagnostics, UK).

For *Yersinia* isolation, 25 g of meat was homogenized and added to 225 ml of phosphate-buffered saline and incubated at 4 °C for up to 3 weeks. After 2 and 3 weeks, the cold-enriched samples were subjected to alkali treatment by adding 0.5 ml of 0.5% KOH into 4.5 ml of cold-enriched samples. The cold-enriched and alkali-treated cultures were streaked onto cefsulodin–irgasan–novobiocin agar (CIN) and incubated at 25 °C for 18–24 h. Presumptive *Yersinia* colonies were identified by biochemical tests using the API 20E (bioMérieux 20100).

2.3. Bacterial sensitivity test to antibiotics

A disk diffusion assay according to the standard protocols (NCCLS, 2003, 2005; CLSI, 2006) was used to determine the susceptibility of *Campylobacter*, *Salmonella* and *Yersinia* isolates to nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (15 µg), streptomycin (30 µg), gentamicin (10 µg), chloramphenicol (30 µg) and amoxicillin (30 µg) (for all isolates); ampicillin (30 µg), colistin (10 µg), spectinomycin (100 µg), erythromycin (15 µg), and neomycin (30 µg) (for *Campylobacter* spp.), ampicillin (30 µg), colistin (10 µg), trimethoprim (15 µg), ceftazidim (100 µg), cephalexin (30 µg) and imipenem (30 µg) (for *Salmonella* spp.); and trimethoprim (15 µg) and cephalotin (30 µg) (for *Yersinia* spp.). The bacterial suspension (in 0.8% NaCl) turbidity were adjusted to 0.5 McFarland, then the suspensions were spread with a sterile cotton swab confluent over the entire

surface of Mueller Hinton agar (Merck, Germany). Standard antibiotic disks (Mast Diagnostics, UK), each containing a specific concentration of an antibiotic, were then applied. After incubation at 37 °C for 18–24 h, the zone of inhibition of growth around each disk was measured in millimeters and zone diameters were interpreted in accordance with CLSI and NCCLS (for *Campylobacter* spp.) guidelines (CLSI, 2006; NCCLS, 2003, 2005).

2.4. Statistical analysis

Statistical analysis of results was performed with SPSS/PC 11.5 software (SPSS Chicago, IL). The Chi-square test and Fisher's exact two-tailed test were used for statistical analysis. A P value <0.05 was used for statistical significance.

3. Results

At least one of the three pathogens was isolated from 208 of the 379 (55%) chicken and beef samples, including 60 (29%) *Salmonella*-, 48 (23%) *Campylobacter*-, and 25 (12%) *Yersinia*-positive samples. Thirty-six percent of the samples contained more than one pathogen. A combination of *Salmonella* and *Yersinia* were isolated from 14 (7%) samples, *Salmonella* and *Campylobacter* from 40 (19%) samples, and *Campylobacter* and *Yersinia* from 10 (5%) samples. All three pathogens were isolated from 11 (5%) samples.

3.1. *Campylobacter*

3.1.1. Prevalence in chicken and beef

One hundred nine (29%) isolates were identified as *Campylobacter* spp. with 83 (76%) identified as *Campylobacter jejuni* and 26 (24%) as *Campylobacter coli*. *Campylobacter* was isolated from significantly more chicken 94 (49.5%) than beef 15 (8%) samples ($P = 0.0001$).

3.1.2. Antimicrobial resistance

Antibiotic sensitivity test was done for 92 (70 *C. jejuni* and 22 *C. coli*) from 109 *Campylobacter* isolates (Table 1). Of 92 isolates, 71% were resistant to nalidixic acid and 47% to ciprofloxacin. In addition, 28% of isolates was resistant to tetracycline, 22.6% to ampicillin, 27% to colistin and 11% to amoxicillin (Table 1). Twelve (13%) of the isolates were susceptible to all of the antibiotics evaluated. Thirty-seven (40.2%) isolates were resistant to at least three antibiotics (multi-drug resistance), and one (1.1%) to six antibiotics with the profile, TET, STR, CIP, NEO, NAL, and AMO (Table 2). Multidrug-resistant isolates were all susceptible to erythromycin. The

Table 1

Number and percentages of antimicrobial-resistant *Campylobacter* isolates from retail chicken and beef.

Antibiotics	<i>C. jejuni</i> N = 70 (%)	<i>C. coli</i> N = 22 (%)	P value	Total N = 92 (%)
AMO	18(25.7)	3(13.6)	0.36	21(23)
AMP	5(7.2)	0(0)	0.33	5(5.5)
TET	22(31.5)	4(18.2)	0.28	26(28)
STR	2(2.8)	0(0)	1.00	2(2.2)
CHL	1(1.4)	0(0)	1.00	1(1.1)
NAL	47(67.1)	18(82)	0.28	65(71)
GEN	0(0)	0(0)	1.00	0(0)
SPT	1(1.4)	1(4.5)	0.42	2(2.2)
ERY	1(1.4)	0(0)	1.00	1(1.1)
COL	22(31.5)	2(9)	0.08	24(26)
NEO	2(2.8)	1(4.5)	1.00	3(3.3)
CIP	34(49)	9(41)	0.62	43(39.5)

AMO; Amoxicillin, AMP; Ampicillin, TET; Tetracycline, STR; Streptomycin, CHL; Chloramphenicol, NAL; Nalidixic acid, GEN; Gentamicin, SPT; Spectinomycin, ERY; Erythromycin, COL; Colistin, NEO; Neomycin, CIP; Ciprofloxacin.

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