Research Note

Loads and antimicrobial resistance of *Campylobacter* spp. on fresh chicken meat in Nueva Ecija, Philippines

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ABSTRACT This study was performed to determine the prevalence and to semiquantify *Campylobacter* spp. on chicken meat samples at 4 selected local wet markets in Nueva Ecija, Philippines, and to determine the antimicrobial resistance patterns of the *Campylobacter* isolates. Out of 120 chicken meat samples, 57 (47.5%) were *Campylobacter* spp. positive. The majority of isolated *Campylobacter* strains were identified as *Campylobacter coli* (54.4%) and 45.6% as *Campylobacter jejuni*. Most of these positive samples (52.6%) showed a very high quantitative *Campylobacter* contamination (most probable number > 2,400/g, lower confidence limit 580/g). For antimicrobial resistance testing, 44 *C. coli*/*jejuni* isolates were tested using the agar disk diffusion method. Out of these, 77.3% were resistant to ampicillin, followed by ciprofloxacin (70.4%), tetracycline (54.6%), erythromycin (20.2%), and gentamicin (11.4%). Of the isolates, 36.4% (n = 16) were resistant to 1 antimicrobial agent, 34.1% (n = 15) were resistance to 3 antimicrobial agents, 13.6% (n = 6) to 2 antimicrobial agents, 9.1% (n = 4) to 4 antimicrobial agents, and 6.8% (n = 3) to all 5 antimicrobial agents tested. Our data demonstrate a high contamination of fresh chicken meat with *Campylobacter* spp. at retail in the Philippines. The detected high *Campylobacter* prevalences and quantitative loads on chicken meat at retail in the Philippines highlight the need to implement efficient intervention measures along the food chain and to encourage sanitary handling of poultry meat.

Key words: *Campylobacter*, chicken meat, prevalence, quantification, antimicrobial resistance

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INTRODUCTION

Campylobacter is one of the most important foodborne zoonotic diseases worldwide. Contaminated poultry and poultry meat is thought to be the major source of human campylobacteriosis. Source attribution models attributed between 58 and 78% of clinical *Campylobacter jejuni* strains to human cases and between 40 and 56% of clinical *Campylobacter coli* strains to chicken sources (Wilson et al., 2008; Sheppard et al., 2009).

Despite the zoonotic importance of this pathogen, only very limited information on *Campylobacter* in animals, food, or humans in the Philippines is available (Magistrado et al., 2001; Baldrias and Raymundo, 2009). Nonetheless, *Campylobacter* prevalence data in poultry meat are available from neighboring countries, such as Thailand and Vietnam, with prevalences of 52 and 31%, respectively (Luu et al., 2006; Vindigni et al., 2007). In a literature survey, Suzuki and Yamamoto (2009) summarized the *Campylobacter* prevalences in poultry meat in Asian countries, ranging from 30% in Vietnam to 82.5% in Turkey.

In many countries, antimicrobial resistances in animal and human *Campylobacter* strains have increased over the years (Hong et al., 2007). In developing countries, where the use of antimicrobial agents in humans and animals is comparably unrestricted, higher rates of enteric infections with antimicrobial-resistant *Campylobacter* spp. were detected (Bungay et al., 2005).

The broiler industry in the Philippines, as in most Asian countries, is more varied and less developed com-

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pared with its Western counterparts. In 2012, around 45% of the total chicken inventories were native or village chickens raised in backyard farms. Chickens grown in commercial farms were broilers and layers, which accounted for 36 and 19%, respectively (BAS, 2012).

Because baseline data for *Campylobacter* contamination on chicken meat are lacking in the Philippines, this study was conducted (i) to determine the prevalence of *Campylobacter* spp. on chicken carcasses, (ii) to describe the semiquantitative load of *Campylobacter* spp., and (iii) to investigate the antimicrobial resistance patterns of the isolated *Campylobacter* spp. strains from chicken meat at retail markets in Nueva Ecija, Philippines.

MATERIALS AND METHODS

Sampling

From January to April 2013, 120 samples of fresh chicken breast meat (with skin) were taken from 4 wet markets, representing 4 districts of the province of Nueva Ecija: Cabanatuan City, San Jose City, Gapan City, and Guimba. Fifteen samples were collected twice from the same chicken meat retail stalls from each wet market using random sampling.

Semiquantification of Campylobacter

For *Campylobacter* detection and semiquantification, ISO 10272–3:2010 (ISO, 2010) was used with some modifications. Briefly, from each sample 15 g of skin were aseptically removed and blended in a stomacher bag with 120 mL of Bolton broth (Oxoid, Basingstoke, UK) and homogenized. Ninety milliliters of the initial suspension was transferred to a 100-mL bottle (corresponding to 10 g of the sample portion). In addition, 10 mL of the initial suspension was transferred to a culture tube and used to create a 10-fold dilution series of up to 10^{-4} by transferring 1 mL to tubes containing 9 mL of Bolton broth. Bolton broths were incubated for 48 h at 42°C in microaerobic conditions (5% O_2, 10% CO_2, 85% N_2; generated by CampyGen, Oxoid). After incubation, 1 loop (approximately 10 μL) of the enrichment was streaked onto modified charcoal cefoperazone deoxycholate agar (Oxoid). Plates were incubated at 42°C for 44 to 48 h in microaerobic conditions. Semiquantitative data are expressed as most probable numbers (**MPN**) per gram in accordance with ISO 10272–3:2010/AC:2011 (ISO, 2011).

Genus and Species Confirmation

For *Campylobacter* confirmation, at least one colony considered to be typical or suspected as being *Campylobacter* spp. was taken, streaked on Columbia blood agar (Oxoid), and incubated at 42°C for 44 to 48 h in microaerobic conditions. Pure cultures were examined for morphology and motility. Isolates were confirmed by biochemical tests (oxidase and catalase test) and gram stained. After DNA extraction, a multiplex PCR was carried out to verify and differentiate *Campylobacter* spp. Primers and protocols were used according to Wang et al. (2002), targeting 23S rRNA from *Campylobacter* spp., *hipO* from *C. jejuni, glyA* from each *C. coli*, *Campylobacter lari*, and *Campylobacter upsaliensis*.

Antimicrobial Susceptibility Test

To investigate the antimicrobial resistance patterns, 23 *C. coli* and 21 *C. jejuni* isolates were tested using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011). *Campylobacter* spp. isolates were microaerobically grown on Columbia blood agar plates (Oxoid) for 24 to 48 h. After incubation colonies were suspended into 1 mL of sterile distilled water until a 0.5 McFarland turbidity was reached. Each suspension was streaked onto a Mueller-Hinton blood agar plate (Difco/BD, Heidelberg, Germany) using a sterile cotton swab (Kirby-Bauer method; Hudzicki, 2013). Five antimicrobial discs were used for sensitivity testing: ampicillin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), and tetracycline (30 μg; all from Oxoid). After placing these disks onto the agar surface, plates were incubated at 42°C for 24 h under microaerobic conditions. After incubation, each disc was examined for absence or presence of a growth inhibition zone by measuring the diameter. The isolates were characterized as susceptible, intermediate, or resistant according to breakpoints for the disk diffusion method for *Campylobacter* spp. provided by Hudzicki (2013).

RESULTS AND DISCUSSION

Prevalence and Semiquantitative Load

The estimated prevalence of *Campylobacter* on chicken meat was 47.5% (57/120; 95% CI: 38.66–56.72). This result is comparable with studies performed in other Asian countries: Vindigni et al. (2007) detected a *Campylobacter* prevalence of 52% on fresh chicken meat at retail in Thailand and Luu et al. (2006) observed a prevalence of 31% on chicken meat in Vietnam. When summarizing prevalence data of different studies performed in Asia, Suzuki and Yamamoto (2009) calculated a *Campylobacter* prevalence of 60.3% on chicken meat in Asian countries.

Of the positive samples, 25% showed a very high quantitative *Campylobacter* load (MPN > 2,400/g, lower confidence limit 580/g; Figure 1). Our data exceeded the quantitative data detected on chicken skin by EFSA (2010) and Chokboonmongkol et al. (2013). These authors detected a lower quantitative contamination of between 1 to 4 log cfu/g with only very small portions of samples containing more than 4 to 5 log cfu/g.

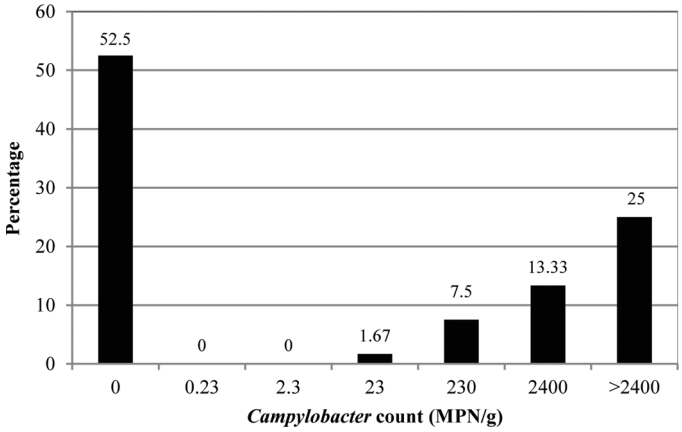

Figure 1. Level of *Campylobacter* concentrations on broiler skin samples (%). MPN = most probable number.

Out of the 57 positive samples, 54.4% (n = 31) were identified to be *C. coli* and 45.6% (n = 26) to be *C. jejuni*. Even though *C. jejuni* is generally regarded as the dominant *Campylobacter* sp. in chicken or poultry (Newell and Fearnley, 2003), several studies described a predominance of *C. coli* in chicken or chicken meat originating from small-scale farming (Meeyam et al., 2004; Padungtod et al., 2006) and organic or free-range production (El-Shibiny et al., 2005; Colles et al., 2008). These authors speculate that the extended rearing periods of free-range and organic birds as well as their specific environmental exposure might explain the predominance of *C. coli* in these cases.

Antimicrobial Resistance Patterns

Altogether, 44 *Campylobacter* isolates (23 *C. coli* and 21 *C. jejuni* isolates) were tested for their antimicrobial resistance patterns. Of these, 77.3% were resistant to ampicillin, followed by ciprofloxacin, 70.4%; tetracycline, 54.6%; erythromycin, 20.2%; and gentamicin, 11.4% (Figure 2). Such high resistance rates to specific antimicrobial agents correspond to previous Asian studies (Padungtod et al., 2006; Baldrias and Raymundo, 2009; Chokboonmongkol et al., 2013). Of the isolates, 36.4% (n = 16) were resistant to 1 antimicrobial agent, 34.1% (n = 15) were resistant to 3 antimicrobial agents, 13.6% (n = 6) to 2 antimicrobial agents, 9.1% (n = 4) to 4 antimicrobial agents, and 6.8% (n = 3) to all 5 antimicrobial agents tested. The most common combination of multidrug resistance was to ampicillin, ciprofloxacin, and tetracycline (34.1%). Comparably, multidrug-resistant *Campylobacter* isolates have been observed already in the Philippines by Baldrias and Raymundo (2009). Resistance of *Campylobacter* isolates is probably related to antimicrobial usage in poultry production, which was observed by Baldrias et al. (2008) based on antimicrobial residue detection in chicken meat in the Philippines.

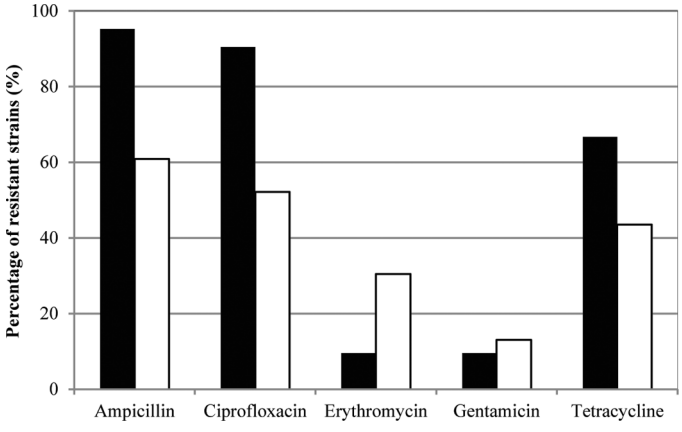

Figure 2. Antimicrobial resistance of *Campylobacter coli* (n = 23) and *Campylobacter jejuni* (n = 21; %). Black columns: *C. jejuni*; white columns: *C. coli*.

Conclusions

Our data demonstrate a high prevalence of *Campylobacter* spp. in fresh chicken meat at retail. Especially the high quantitative load of a substantial share of the investigated samples must be of concern. To reduce the prevalence and quantitative load of *Campylobacter* on chicken meat, different intervention measures along the food chain must be implemented: establishment of strict biosecurity measures at the farm level and implementation of good and efficient intervention measures at slaughterhouses to minimize fecal contamination of broiler skin and reduce cross-contamination. Awareness should be raised for the safe handling of poultry meat due to the presence of high *Campylobacter* numbers on fresh chicken meat.

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