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Prevalence, Numbers and Antimicrobial Susceptibilities of *Salmonella* Serovars and *Campylobacter* spp. in Retail Poultry in Phnom Penh, Cambodia

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ABSTRACT. *Salmonella* and *Campylobacter* are common bacterial pathogens associated with human gastro-enteritis; and raw poultry is considered to be an important source of these bacteria. To evaluate whether the *Salmonella* serovars and *Campylobacter* spp. bacteria could be monitored for the purpose of microbial presence, enumeration and antimicrobial resistance in raw poultry, 152 poultry carcasses were randomly selected from 10 markets in retail outlets of Phnom Penh during March 2006 to February 2007. The majority of poultry samples was contaminated by *Salmonella* serovars (88.2%) and *Campylobacter* spp. (80.9%). A very high contamination of *Salmonella* was found at 3–4 $\log_{10}$ CFU/g for 22.4% of samples and of *Campylobacter* at 7–8 $\log_{10}$ CFU/g for 1.3% of samples. Fifty nine different *Salmonella* serovars contaminated 134 poultry carcasses; five most prevalent serovars covered 29.1% of serovars isolates (Anatum, Typhimurium, Corvallis, Stanley and Enteritidis). Three *Campylobacter* species contaminating 123 raw poultry were *Campylobacter jejuni* (50.0%), *Campylobacter coli* (29.0%) and *Campylobacter lari* (21.0%). High antibiotic resistance percentages were found among *Salmonella* serovars and *Campylobacter* spp. isolates. This study revealed that raw poultry at the retail outlets in Phnom Penh markets are contaminated with high prevalences of food-borne pathogens, and communicating the importance of minimizing this risk in reducing human infections.

KEY WORDS: antimicrobial susceptibility, *Campylobacter*, poultry, prevalence, *Salmonella*.

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Salmonella and *Campylobacter* are both the most important food-borne diseases and cause substantial medical and economic burdens worldwide. In developing countries, investigations have shown that infection caused by *Campylobacter* spp. may be as serious as those by *Salmonella* serovars, both in frequency and severity symptoms [3]. Poultry is one of the principal reservoirs of non typhoid human *Salmonella* infection and causes potential of food-poisoning hazards [5, 14]. *Campylobacter jejuni* and *Campylobacter coli* infecting also poultry carcasses are major causes of gastroenteritidis in human [7, 17]. To prevent poultry carcass contaminations, it is crucial to control *Salmonella* serovars 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 outlets [3]. Because of this, a number of actions have been taken to reduce the prevalence of *Salmonella* and *Campylobacter* with public health significance in food-producing animals. Quantitative microbiology risk assessment is still hampered by the lack of quantitative data. The generation of appropriate data with high sensitivity is a challenge for microbiologists since currently used bacteriological quantitation methodologies are laborious. Furthermore, quantitative *Salmonella* data for food associated with severe outbreaks have shown that the type of food plays a major role in the severity of illness. *Salmonella* in fatty food may have an advantage during passage through the acidic environment of the stomach to the intestine, where the cells become invasive regardless the damage caused by the acids.

In Cambodia, very little is known regarding the occurrence of food borne disease caused by enteric bacteria. For this, the authors processed to evaluate prevalence's, numbers and antimicrobial susceptibilities of *Salmonella* serovars and *Campylobacter* spp. in retail outlets of Phnom Penh markets during one year period.

MATERIALS AND METHODS

Samples: Between March 2006 to February 2007, 152 poultry carcasses were collected from retail outlets of 10 markets in Phnom Penh city of Cambodia. Poultry were slaughtered directly in these markets sites. Every week, three samples were selected from each fixed retail outlet within three different markets, and another week, three other markets will be interested. The random was processed like this along 10 markets during one year period. Neck skin of poultry carcasses were selected, because the small hair of poultry neck skin retained micro-organisms.

Isolation and identification of Salmonella: Culture and isolation of *Salmonella* was conducted using standard method ISO 6579 as previously described [11]. Transparent well-isolated colonies with black center typical *Salmonella* morphology were collected from selective media and identified by using biochemical reactions. Isolates with typical *Salmonella* were confirmed to be *Salmonella* serovars based on detection of somatic and flagella antigens.

Enumeration of Salmonella: A semi-quantitative approach using modified semisolid Rappaport Vassiliadis (MSRV) agar was applied [18] by practice successive dilutions and by aspired 0.5 m*l* of aliquot (1/10) from the 1st

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serial to 4th serial of wells. After incubation the microplates at 37°C for 20 hr, 20 μl of each dilution was peripheral transferred to the 2nd microplate to corresponding wells filling with 2 m*l* of MSRV media. The 2nd microplate was incubated at 41.5°C for 24–48 hr. Wells of agar semisolid-MSRV presenting a range of migration with a discoloration were presumed to be positive. The positive or doubtful wells were cultured onto Hektoen (Difco) agar plate at 37°C for 20 hr, for a confirmation of positive *Salmonella* as above. For the *Salmonella* enumeration, the positive and negative result for each sample should be seized in the mask of MNP calculator, the weight of each sample and serials of dilution rates included in the test from 1st to 4th well were noted as 0.2 g at 1/10, 0.04 g at 1/5, 0.008 g at 1/25 and 0.0016 g at 1/125, a number 1 was noted for a positive well and a number 0 for a negative well.

Isolation and identification of Campylobacter: Culture of *Campylobacter* was conducted using standard method ISO 10272–1 as previously described [15] and characteristic colonies (grayish on Karmali and grey on CCDA) were suspended in Brucella broth; *Campylobacter* bacterium was identified as curved bacterium with Gram (–) coloration and a spin movement under the microscope. Biochemical identifications were done on TSI agar (Difco) slant tubes, oxydase test, catalase test, sensibility to nalidixic acid and cefalotin disks, hydrolysis of hippurate and growth test at 25°C in Brucella broth. The sensitivity to nalidixic acid differentiated *C.lari* from *C. jejuni* and *C. lari*.

Enumeration of Campylobacter: The method ISO/CD 10272–2 was used to enumerate *Campylobacter* as previously described [10] and a loopful of suspect positive aliquot was cultured on Colombia plates (Oxoid) in microaerophilic atmosphere at 42°C during 24 hr. Biochemical identifications of *Campylobacter* spp. were done identically as above.

Antimicrobial susceptibility test: All *Salmonella* and *Campylobacter* isolates were tested for antimicrobial susceptibilities by the disk diffusion method [16]. The following antimicrobials were tested at the indicated concentration (in μg/disk except where specified) for *Salmonella* isolates: amoxicillin, amoxicillin/clavulanic acid, ticarcillin, cefalotin, cefoxitin, cefotaxim, gentamicin, streptomycin, chloramphenicol, sulfonamide, cotrimoxazol, nalixidic acid, ciprofloxacin and tetracycline. For *Campylobacter* isolates, the disks tested were amoxicillin, cefalotin, gentamicin, erythromycin, azithromycin, nalidixic acid and ciprofloxacin.

The minimum inhibitory concentrations (MIC) of the antimicrobial agents against isolates and the breakpoints were determined by NCCLS criteria and read by Osiris system (Biorad).

Statistical analysis: The prevalence of *Salmonella* and *Campylobacter* was calculated by using Microsoft Excel. The general formula for *Salmonella* count (MNP method) and *Campylobacter* spp. colonies count using for plates contained between 15 to 150 colonies were applied.

RESULTS

Salmonella were isolated from 134 (88.2%) out of 152 samples processed with 201 isolates, whereas *Campylobacter* were isolated from 123 (80.9%) samples including 139 isolates.

Different quantitative contaminations of *Salmonella* were displayed by 34 samples (22.4%) at 3–4 $\log_{10}$CFU/g, 56 samples (36.8%) at 2–3 $\log_{10}$CFU/g, 32 samples (21.1%) at 1–2 $\log_{10}$CFU/g, and 12 samples (7.9%) at 0–1 $\log_{10}$CFU/g (Fig. 1).

Among 134 positive samples, seven samples harbored four different species of *Salmonella* (5%), 12 samples of 3 different species (9%), 49 samples of 2 different species (37%) and 66 samples of one specie (49%).

Fifty nine *Salmonella* serovars from positive samples are listed in the Table 1. The most prevalent serotypes are *Salmonella* Anatum, *Salmonella* Typhimurium, *Salmonella* Corvallis, *Salmonella* Stanley and *Salmonella* Enteritidis that covered 29.1% of all isolated serovars.

The contamination of raw poultry by *Campylobacter* spp. was found successively from high to low quantitative rates: 2 samples (1.3%) at 7–8 $\log_{10}$CFU/g, 6 samples (3.9%) at 6–7 $\log_{10}$CFU/g, 33 samples (21.7%) at 5–6 $\log_{10}$CFU/g, 50 samples (32.9%) at 4–5 $\log_{10}$ CFU/g, 25 samples (16.4%) at

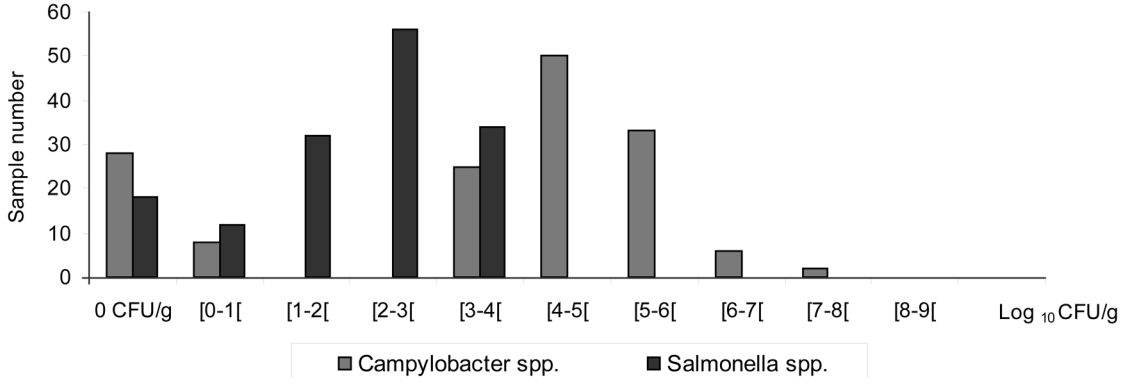

Fig. 1. Quantitative contaminations of *Salmonella enterica* serovars and *Campylobacter* spp. in raw poultry.

Table 1. *Salmonella* (*S.*) serovars isolated from raw poultry

Salmonella serovars	201 isolates	%
S. Anatum	13	6.4
S. Typhimurium	13	6.4
S. Corvallis	12	5.9
S. Stanley	11	5.5
S. Enteritidis	10	4.9
S. Derby	9	4.5
S. Weltevreden	9	4.5
S. Albany	8	3.9
S. Hvittingfoss	8	3.9
S. Newport	8	3.9
S. London	7	3.5
S. Braenderup	6	2.9
S. Lexington	6	2.9
S. Bovismobificans	4	1.9
S. Nakuru	4	1.9
S. Ohio	4	1.9
S. Paratyphi B	4	1.9
S. Rissen	4	1.9
S. Schleissheim	4	1.9

Other serovars less than 4 isolates: Amsterdam, Altona, Atakpame, Bareilly, Be, Biafra, Bradford, Chailey, Clackamas, Djugu, Dublin, Eschberg, Give, Hadar, Hayindogo, Hessarek, Indiana, Ituri, Istoria,Javiana, Kentucky, Lamberhurst, Loubomo, Mbandaka,Orientalis, Reading, Regent, Sandow, Saintpaul, Sarajane, Schwarzengrund, Sinchew, Sinstorf, Thompson, Tsevie, Tyresoe Virchow, Uganda, Wansworth.

3–4 log $_{10}$ CFU/g and 8 (5.3%) at 0–1 log$_{10}$ CFU/g (Fig. 1).

One hundred thirty nine isolates of *Campylobacter* recovered from three species contaminated poultry carcasses; *Campylobacter jejuni* was more frequently isolated (50.0%) than *Campylobacter coli* (29.0%) or *Campylobacter lari* (21.0%).

Table 2 shows the results of antimicrobial susceptibility tests for *Salmonella* serovars circulating among raw poultry in Phnom Penh markets. All *Salmonella* isolates were sensitive to cefotaxim and cefoxitin. About 23.1–53.8% of *Salmonella* Anatum presented high resistant rates to six antimicrobials (amoxicillin, cefalotin, cotrimoxazol, nalidixic acid, sulfonamid and tetracycline). In other hand *Salmonella* Typhimurium were resistant to four antimicrobials from 15.4–23.1% (amoxicillin, nalidixic acid, sulfonamid and tetracycline). *Salmonella* Corvallis presented high proportions of resistance to nalidixic acid, sulfonamid and tetracycline (respectively 42.0, 75.0 and 75.0%). *Salmonella* Stanley displayed a resistance only to tetracycline (90.0%). The resistance of *Salmonella* Enteritidis was important and varied from 10.0–90.0% to amoxicillin, ticarcillin, nalidixic acid, streptomycin, chloramphenicol and tetracycline.

The antimicrobial resistance was apparent for *Campylobacter jejuni* (97.1%), *Campylobacter coli* (97.5%) and *Campylobacter lari* (96.7%) displaying to cefalotin. About 90.0%, 69.6% and 15.0% of *Campylobacter lari, Campylobacter jejuni* and, *Campylobacter coli* were resistant to nalidixic acid. Three antimicrobial agents (amoxicillin, azithromycin and erythromycin) were completely inhibiting by *Campylobacter coli*. Otherwise, *Campylobacter jejuni* was sensitive to gentamicin (Table 3).

DISCUSSION

The present study conducted over 1 year period shows high prevalences and numbers of 2 major enteric bacteria contaminating raw poultry in retail outlets among 10 Phnom Penh city markets. These bacteria resulted from a cross contamination of the kitchen environment bacteria and direct hand-to-mouth exposure of enteric pathogens [6]. The prevalence (88.2%) of *Salmonella* serovars in Cambodia from poultry carcasses was at the same level as the prevalence of *Salmonella* in other developing countries, 72.0% in Thailand from retail chicken meat samples and 80.0% of poultry in open markets [1], although only 4.6% of farming ducks in

Table 2. Prevalence of antimicrobial resistance in five most prevalent *Salmonella* (*S.*) serovars and other serovars isolated from raw poultry[a)]

Antimicrobial	Resistant number (%)					
	S. Anatum n=13	*S.* Typhimurium n=13	*S.* Corvallis n=12	*S.* Stanley n=11	*S.* Enteritidis n=10	Other serovars n=142
Amoxicillin	3 23.1	2 15.4	1 8.3	0 0	9 90.0	19 13.4
Amox./clav. acid	0 0	0 0	0 0	0 0	0 0	1 0.7
Cefalotin	3 23.1	0 0	0 0	0 0	0 0	0 0
Cefotaxim	0 0	0 0	0 0	0 0	0 0	0 0
Cefoxitin	0 0	0 0	0 0	0 0	0 0	0 0
Chloramphenicol	0 0	0 0	1 8.3	0 0	1 10.0	10 7.0
Ciprofloxacin	0 0	0 0	1 8.3	0 0	0 0	4 2.8
Cotrimoxazol	3 23.1	0 0	0 0	0 0	1 10.0	10 7.0
Gentamicin	0 0	0 0	0 0	0 0	0 0	2 1.4
Nalidixic acid	7 53.8	3 23.1	5 41.7	0 0	8 80.0	24 16.9
Streptomicin	0 0	1 7.7	0 0	0 0	6 60.0	1 0.7
Sulfonamid	3 23.1	2 15.4	9 75.0	0 0	1 10.0	12 8.5
Tetracycline	7 53.8	2 15.4	9 75.0	1 9.0	1 10.0	23 16.2
Ticarcillin	1 7.7	0 0	1 8.3	0 0	9 90.0	21 14.8

a) Antimicrobial susceptibility testing was performed according to NCCLS guidelines [16]. *Escherichia coli* ATCC 25922 were used as the quality control organism for *Salmonella* serovars.

Table 3. Prevalence of antimicrobial resistance of *Campylobacter* (*C.*) *jejuni*, *C. coli* and *C. lari* isolated from raw poultry in markets[a)]

Antimicrobial	Resistant number (%)					
	C. *jejuni* n=69		*C. coli* n=40		*C. lari* n=30	
Amoxicillin	9	13.0	0	0	6	20.0
Azithromycin	1	1.4	0	0	4	13.3
Cefalotin[b)]	67	97.1	39	97.5	29	96.7
Ciprofloxacin	14	20.3	3	7.5	19	63.3
Erythromycin	2	2.9	0	0	4	13.3
Gentamicin	0	0	1	2.5	1	3.3
Nalidixic acid	48	69.6	6	15.0	27	90.0

a) Antimicrobial susceptibilities testing was performed according to NCCLS guidelines [16]. *C. jejuni* ATCC 33560 was used as the quality control organism for *Campylobacter*.

b) Cefalotin resistance is one a most key properties identify these three species, and Nalidixic acid sensibility differentiated *C. lari* from *C. jejuni* and *C. coli*.

Taiwan were positive for *Salmonella* [20]. In developed countries, the level of *Salmonella* contamination of raw chicken was 30.8% in poultry layer feces in North Carolina (U.S.A.) [12], a mean of prevalence was 22.4% in reproductive laying hens in Poland, over a 5 year period (2001–2005) [19] and 13.4% of laying hens were positive in the Netherlands [21].

The numbers of *Salmonella* in our study varied from less than 1 to 4 $\log_{10}$ CFU/g for 134 samples. Nevertheless, no spoilage was observed in the poultry meat before processing and it was thought that competition among the micro-organisms in the poultry meat might suppress the growth of *Salmonella* cells [8]. A previous study showed that inoculated 10^5 CFU/g of *Salmonella* cells in minced meat, they grew to a high population level (10^9 CFU/g) [13]. Another study in U.S.A. showed also same range of *Salmonella* number which varied from <1.00 to 3.76 $\log_{10}$ CFU/g in layer feces hens [12].

A high prevalence of *Campylobacter* spp. was calculated in the study (80.9%). In Thailand a high prevalence rate of *Campylobacter jejuni* was also observed from broilers flocks that correlated with the results of our study (65.0%) [4]. In the Netherlands a previous study showed that *Campylobacter* spp. were positive for 27.1% of chicken broilers [21]. Our results showed high quantitative numbers of *Campylobacter* spp. in Cambodian markets that varied from <1–8 $\log_{10}$CFU/g in poultry carcasses. In Europe *Campylobacter* spp. were found in moderate rates, from less than 1–4.5 $\log_{10}$CFU/g in Belgium chicken meat [9] and in Italian broilers, the mean rates varied from 3.93–6.13 $\log_{10}$CFU/g [14].

High multiple antimicrobial resistance profiles were observed for *Salmonella* Anatum, *Salmonella* Typhimurium, *Salmonella* Corvallis, *Salmonella* Enteritidis and other *Salmonella* serovars to amoxicillin (8.3–90.0%), nalidixic acid (16.9–80.0%), sulfonamide (8.5–75.0%) and tetracycline (15.4–90.0%). Only *Salmonella* Stanley was resistant to tetracycline in a moderate rate (9.0%), all other antimicrobials tested were completely inhibiting by *Salmonella* Stanley. The same level of resistance of *Salmonella* Typhimurium to nalidixic acid (23%) was observed in a study performing in France [2]. Our results on the resistance of *Salmonella* Enteritidis to nalidixic acid were higher than what was seen in Europe in raw poultry (15.0%) [2].

Results of this study show important information regarding the contamination of *Salmonella* and *Campylobacter* spp in poultry among Phnom Penh markets, and the dissemination of resistance of these bacteria to major antibiotics confirming the crucial role of raw animal as the source of resistance to antimicrobial spread. Thus, it is important to increase the awareness of veterinary authorities to promote the hygiene practices at the slaughter of poultry within Cambodian markets. It is also recommended to monitor the poultry farming as a preventive measure to avoid the micro-organism development in the initial chain of poultry consumption.

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