Asian Pac J Trop Biomed 2017; 7(7): 670–674

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.07.002

Antibiotic susceptibility and molecular characterization of resistance genes among *Escherichia coli* and among *Salmonella* subsp. in chicken food chains

Yith Vuthy[1*], Kruy Sun Lay[1], Heng Seiha[1], Alexandra Kerleguer[1], Awa Aidara-Kane[2]

[1]*Institut Pasteur, Phnom Penh, Cambodia*

[2]*World Health Organization, Geneva, Switzerland*

ARTICLE INFO

Article history:
Received 29 May 2017
Accepted 19 Jun 2017
Available online 12 Jul 2017

Keywords:
Chicken food chains
Escherichia coli
Salmonella
Resistance gene
Susceptibility

ABSTRACT

Objective: To investigate the occurrence of resistance genes among *Escherichia coli* (*E. coli*) and *Salmonella* subsp. isolated in chicken food chains in Phnom Penh, 2012–2013.
Methods: Six hundred eighty two *E. coli* and 181 *Salmonella* Albany, Corvallis, and Kentucky strains were examined for susceptibilities to eight antimicrobials and following resistance genes were identified by PCR: bla_{Tem}, *StrA*, *aadA*, *sul1*, *sul2*, *gyrA*, *Tet (A)*, and *Tet (B)*.
Results: *E. coli* presented high resistances to tetracycline, amoxicillin, and sulfamethoxazole (63.1%–76.1%). *Salmonella* Albany and *Salmonella* Kentucky traduced high resistance percentages to amoxicillin, tetracycline, sulfamethoxazole, and nalidixic acid (84.6%–100%). Among amoxicillin-resistant isolates, bla_{Tem} genes were observed for 62% of *E. coli* isolates and 20% of 65 *Salmonella* Kentucky. The *StrA* gene was prevalent in 36% of 331 aminoglycoside-resistant *E. coli* and 90% of 40 aminoglycoside-resistant *Salmonella* Corvallis. The *sul2* gene was predominant among sulfamethoxazole-resistant isolates, for 56% of 431 *E. coli* and 53% of 66 *Salmonella* Corvallis; the *sul1* gene was observed in 54% of *Salmonella* Albany. The *Tet (A)* resistance gene was prevalent in *E. coli* (86%), *Salmonella* Corvallis (82%), *Salmonella* Kentucky (84%). High percentages of *gyrA* genes observed among nalidixic-acid resistant *E. coli* (91%), *Salmonella* Albany (92%), *Salmonella* Corvallis (75%) and *Salmonella* Kentucky (85%).
Conclusions: Important occurrences of resistance gene were observed among *E. coli* and *Salmonella* in chicken food chains in Cambodia.

1. Introduction

Escherichia coli (*E. coli*) is a Gram-negative bacterium and part of the commensal gut flora of humans, chickens and other livestock animals. It can, however, be pathogenic in all of these species. *E. coli* is of clinical importance due to its ability to initiate and establish various kinds of infections [1]. It is one of the most frequently isolated organisms from different clinical presentations of diarrhea in humans, including traveler's diarrhea [2]. Moreover, *E. coli* is one of the main causes of nosocomial infections in humans, pathogenic strains being most commonly associated with urinary tract infections [3]. *Salmonella enterica* is also a Gram-negative bacterium. These typhoid and non-typhoidal *Salmonella* species are zoonotic agents, and are predominantly associated with foodborne infections in humans [4]. Salmonellosis in humans is generally contracted through the consumption of contaminated and poorly handled food, non-typhoid salmonellosis remains widespread, because of food contamination or asymptomatic carriage including foods of animal origin (mainly meat, chicken, and eggs) [5]. The clinical course of human non-typhoid salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting. Although largely treatable, non-typhoid fevers may be severe in about 10%–15% of cases [6], and continue to be important causes of illness and death, particularly among children and adolescents in Southeast Asia [7].

In recent years, frequent misuse of antimicrobials contributed to increase high prevalence of resistance to the common

*Corresponding author: Yith Vuthy, Institut Pasteur, Phnom Penh, Cambodia.
Tel: +855 012 799 508
Fax: +855 23 725 606
E-mail: yvuthy@pasteur-kh.org (Y. Vuthy).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

antimicrobial used in humans among *E. coli* [8] and *Salmonella enterica* that have been isolated in hospitalized patients in Cambodia and in chicken meat products [9,10]. Antimicrobial agents are currently used for three main reasons: to treat infections in humans and animals, prophylactically in humans, and animals; and subtherapeutically in food animals as growth promoters. When persistent antibiotic use becomes the norm in both human and animal medicine, there is an increased pressure for developing and maintaining new resistance genes that can be shared among bacterial populations [11].

The study investigated the antibiotic susceptibility of *E. coli* isolated from chicken slaughter chains in Phnom Penh (PP) markets and most representative serovars of *Salmonella* isolated, *Salmonella* serovars Albany, Corvallis and Kentucky, from slaughter chains in PP markets and from farming chicken, to eight antimicrobials including, amoxicillin, amoxicillin/clavulanic acid, cephalothin, gentamicin, streptomycin, sulfamethoxazole, nalidixic acid, and tetracycline. The eight resistance genes bla_{Tem}, *StrA*, *aadA*, *sul1*, *sul2*, *gyrA*, *Tet (A)*, and *Tet (B)* were characterized.

2. Materials and methods

2.1. Samples design in chicken food chain

From February 2012 to October 2013, a total of 762 samples were collected comprising 80 chicken feces from two large chicken farms selected in neighboring areas of PP city, fecal samples were regularly selected from live chickens by the district veterinary services of PP, additionally with, 82 chicken caecae, 440 chicken neck skins, 80 rinse water and 80 chopping boards samples selected inside chicken slaughter chains and were collected from two PP live bird markets, Orussey and Dem Kor.

All chicken stalls in the two markets were listed, on Tuesday each week, the choice of stall and sample (*e.g.* caecae, neck skin, rinse water, and chopping board) was randomly selected using a random number table. At the markets, caecal swabs were collected from chickens and neck skin samples were taken from carcasses. At the point of sale, water samples used to wash chicken carcasses were collected into sterile bottles and chopping boards used to chop carcasses were also sampled by swabbing 100 cm^2 of the surface.

2.2. Microbiology examination

All samples were transported from the sites to the Institut Pasteur du Cambodge laboratory within an hour. Chicken feces, chicken meat, environmental samples were processed immediately upon arrivals. *E. coli* identifications in meat samples used Afnor validation method no Biorad-Rad 07/01-07/93 BRD 0717-12/04, and *Salmonella* subsp. was isolated and identified according to the standard NF EN ISO 6579, 2002 method.

2.3. E. coli and Salmonella organisms

A total of 682 market slaughter chains samples were positive with *E. coli*. Another total of 376 *Salmonella* isolates selected from 762 samples of above study 2012–2013, those three serovars [*Salmonella* Albany (n = 26), *Salmonella* Corvallis (n = 86) and *Salmonella* Kentucky (n = 69)] represented 48.1% and others 52 serovars represented 51.9% of *Salmonella* strains.

2.4. Susceptibility testing

All *E. coli* and *Salmonella* Albany, Corvallis, and Kentucky isolates were tested for their resistance to eight antimicrobials including, amoxicillin, amoxicillin/clavulanic acid, cephalothin, gentamicin, streptomycin, sulfamethoxazole, nalidixic acid, and tetracycline, following the recommendations of the National Committee for Clinical Laboratory Standards guidelines 2014. Strains were considered multiple drug resistant isolates (MDR) when these strains were resistant to at least three antibiotics. *E. coli* ATCC25922 was used as reference strain for the susceptibility test.

2.5. Identification of resistance genes

PCR was used to detect the presence of eight resistance genes; primers are described in Table 1 with published references. Most primers were designed to differentiate the specific gene sequences of interest; the only exceptions were the bla_{Tem} primers, which amplified the entire family of bla_{Tem} gene. As positive controls during PCR processes, reference fragments of resistance genes were obtained from the Danish National Food Institute (DTU, WHO collaborative center).

2.6. Sequencing and alignment of fragments

Using the same primers as in the PCR analysis, amplicons were sequenced to verify the identity of the gene products in all *E. coli* and *S.* Albany, *S.* Corvallis, *S.* Kentucky to evaluate bla_{Tem}, *StrA*, *aadA*, *sul1*, *sul2*, *gyrA Tet (A)*, and *Tet (B)* genes. The resulting nucleotide sequences were compared to nucleotides fragments obtained from the GenBank databases (NCBI). After sequencing PCR products of eight resistance genes (bla_{Tem}, *StrA*, *aadA, sul1*, *sul2, gyrA, Tet (A)*, and *Tet (B)*) in *E. coli* and in *Salmonella* Albany, Corvallis, and Kentucky, alignments using Logiciels BioEdit and MEGA 6 revealed a homology from 90% to 95% of all PCR fragments with reference sequences.

3. Results

For 682 *E. coli* isolated from chicken slaughter chains in markets, 519 (76.1%) were resistant to tetracycline, 507 resistant to amoxicillin (74.3%) and 431 resistant to sulfamethoxazole (63.1%). In total 406 *E. coli* (59.5%) were MDR strains resistant to at least three different classes of antimicrobials in the panel of drugs tested (Table 2).

Table 2 describes also the phenotypic resistance patterns of *S.* Albany, *S.* Corvallis, and *S.* Kentucky. Among 26 *S.* Albany in the study, 25 were MDR (96.1%) with 25 isolates resistant to amoxicillin and nalidixic acid (both 96.1%), 22 isolates resistant to tetracycline (84.6%), and all *S.* Albany resistant to sulfamethoxazole (100%). Among *S.* Corvallis (n = 86), 41 isolates were MDR strains (47.6%) with 40 isolates resistant to streptomycin (46.5%), 66 resistant to sulfamethoxazole (76.7%), and 68 isolates resistant to tetracycline (79.0%). Sixty-seven MDR strains (97.1%) were observed among *Salmonella* Kentucky (n = 69), with 67 isolates resistant to tetracycline and nalidixic acid (both 97.1%), 65 isolates resistant to amoxicillin (94.2%), and 64 strains resistant to sulfamethoxazole (92.7%).

Table 3 shows the distribution of resistance genes among phenotypically-resistant *E. coli* isolated from markets. Among

Table 1
List of oligonucleotides primers used for PCR of resistance genes for *E. coli* and *Salmonella* subspecies.

Drug class	Gene	Oligonucleotides primers	Fragment size (bp)	Annealing temp.(°C)	Ref
Beta-lactam	Bla_{Tem}	(F) ACCAATGCTTAATCAGTGAG (R) GCGGAACCCCTATTTG	963	50	[12]
Aminoglycoside	*StrA*	(F) CCAATCGCAGATAGAAGGC (R)CTTGGTGATAACGGCAATTC	548	58	[13]
	aadA	(F) GTGGATGGCGGCCTGAAGCC (R) AATGCCCAGTCGGCAGCG	528	58	[14]
Sulfamethoxazole	*Sul1*	(F) GTGACGGTGTTCGGCATTCT (R) TTTACAGGAAGGCCAACGGT	668	58	[15]
	Sul2	(F) GGCAGATGTGATCGACCTCG (R) ATGCCGGGATCAAGGACAAG	405	58	[15]
Nalidixic acid	*gyrA*	(F) ATGAGCGACCTTGCGAGAGAAATTACACCG (R) TTCCATCAGCCCTTCAATGCTGATGTCTTC	630	58	[16]
Tetracycline	*Tet(A)*	(F) GCTACATCCTGCTTGCCTTC (R) CATAGATCGCCGTGAAGAGG	210	58	[12]
	Tet(B)	(F) TTGGTTAGGGGCAAGTTTTG (R) GTAATGGGCCAATAACACCG	659	5	[12]

Table 2
Number of resistant *E. coli* and *Salmonella* Albany, Corvallis, and Kentcuky belonging to chicken food chains [*n* (%)].

Antimicrobial agent	*E. coli*	*S.* Albany	*S.* Corvallis	*S.* Kentucky
Amoxicillin	507 (74.3)	25 (96.1)	2 (2.3)	65 (94.2)
Amoxicillin/clavulanic acid	15 (2.1)	0 (0.0)	2 (2.3)	0 (0.0)
Cephalothin	13 (1.9)	0 (0.0)	2 (2.3)	0 (0.0)
Gentamicin	17 (2.4)	0 (0.0)	3 (3.4)	0 (0.0)
Streptomycin	314 (46.0)	2 (7.6)	40 (46.5)	3 (4.3)
Sulfamethoxazole	431 (63.1)	26 (100)	66 (76.7)	64 (92.7)
Nalidixic acid	355 (52.1)	25 (96.1)	8 (9.3)	67 (97.1)
Tetracycline	519 (76.1)	22 (84.6)	68 (79.0)	67 (97.1)
MDR isolates	406 (59.5)	25 (96.1)	41 (47.6)	67 (97.1)

n: number of resistant isolates; MDR strains, resistant to at least three different classes of antimicrobials.

Table 3
Occurrence of resistance genes among *E. coli,* and *Salmonella* serovars Albany, Corvallis, and Kentucky.

Antimicrobial agents	Genes tested	*E. coli*	*S.* Albany	*S.* Corvallis	*S.* Kentucky
Amoxicillin	bla_{TEM}	312 (62)	2 (8)	0	13 (20)
	Unknown	195 (38)	23 (92)	2 (100)	52 (80)
Aminoglycoside	*StrA*	120 (36)	0	36 (90)	1 (33)
	aadA	56 (17)	0	1 (2)	1 (33)
	StrA + aadA	128 (38)	0	0	0
	Unknown	29 (7)	2 (100)	3 (8)	1 (33)
Sulfamethoxazole	*Sul1*	14 (3)	14 (54)	0	25 (39)
	Sul2	241 (56)	1 (4)	35 (53)	2 (3)
	Sul1 + Sul2	28 (7)	0	0	1 (2)
	Unknown	148 (34)	11 (42)	31 (47)	36 (56)
Nalidixic acid	*GyrA*	322 (91)	23 (92)	6 (75)	57 (85)
	Unknown	33 (9)	2 (8)	2 (25)	10 (15)
Tetracyclines	*Tet(A)*	446 (86)	5 (22)	56 (82)	56 (84)
	Tet(B)	38 (7)	0	0	0
	Tet(A) + Tet(B)	8 (2)	0	0	0
	Unknown	28 (5)	17 (78)	12 (18)	11 (16)

Data are expressed as *N*, RG (%); *N*: Number of resistant isolates; RG: Number of positive isolates with resistant gene; (%): percentage of resistant isolates.

507 amoxicillin resistant *E. coli*, 312 (62%) gave positive amplicons for the bla_{Tem} gene. One hundred and twenty of 331 aminoglycoside-resistant *E. coli* carried the *StrA* gene (36%), 56 the *aadA* gene (17%) and 128 both the *StrA* + *aadA* gene (38%) (Table 3). Fourteen of 431 sulfamethoxazole-resistant isolates carried the *sul1* gene (3%), 241 the *sul2* gene (56%), and 28 both the *sul1* + *sul2* gene (7%). Three hundred twenty two of 355 *E coli* resistant to nalidixic acid carried the *GyrA* gene (91%). Four hundred and forty six of 519 tetracycline-resistant isolates contained the *Tet (A)* gene (86%), 38 the *Tet (B)* gene (7%), and 8 the *Tet (A)* + *Tet (B)* gene (2%).

The distribution of resistance genes among phenotypic-resistant *Salmonella* serovars isolated from chicken food chains is showed in Table 3. Only two of 25 *S.* Albany were phenotypically-resistant to amoxicillin harbored the bla_{Tem} gene (8%). The genes responsible for the aminoglycoside resistance profile for two *S.* Albany were not detected. Fourteen of 26 sulfamethoxazole-resistant *S.* Albany isolates contained the *sul1* gene (54%) and one isolate the *sul2* gene (4%), five of 22 tetracycline-resistant *Salmonella* Albany contained the *Tet (A)* gene (22%), and 23 of 25 *Salmonella* Albany resistant to nalidixic acid carried the *GyrA* gene (92%).

Among *Salmonella* Corvallis, the genes responsible for two amoxicillin-resistant isolates were not detected. Thirty-six of 40 aminoglycoside-resistant *Salmonella* Corvallis yielded amplicons for the *StrA* gene (90%) and one isolate for the *aadA* gene (2%). Among 66 *Salmonella* Corvallis resistant to sulfamethoxazole, only 35 strains presented amplicons for the *sul2* gene (53%). Six of eight *Salmonella* Corvallis strains resistant to nalidixic acid yielded the *gyrA* gene (75%). Fifty-six out of 68 tetracycline-resistant *Salmonella* Corvallis carried the *Tet (A)* gene (82%).

In *Salmonella* Kentucky, 13 of 65 amoxicillin-resistant strains yielded the bla_{Tem} gene (20%), and one isolate of *Salmonella* Kentucky resistant to aminoglycoside presented one amplicon of the *StrA* gene (33%), and another isolate traduced one amplicon of the *aadA* gene (33%). Among 64 isolates resistant to sulfamethoxazole, 25 carried *sul1* (39%), two were positive for *sul2* (3%), and one isolate carried both the *sul1* + *sul2* gene (2%). Fifty seven of 67 nalidixic-acid resistant isolates to nalidixic acid antibiotic carried the *gyrA* gene (85%). Fifty-six of 67 tetracycline-resistant *Salmonella* Kentucky isolates contained the *Tet (A)* gene (84%).

4. Discussion

Our study presents a moderate percentage of MDR strains among *E. coli* strains isolated from chicken food chains in Phnom Penh markets (59.5%) compared with *E. coli* MDR strains isolated (85.3%) from chicken farms, in Tien Giang province, South Vietnam, where the assessment was conducted during same period [8]. This high MDR prevalence in *E. coli* in Vietnam samples might due direct boot swab samples of household and small-scale chicken farms. Tetracycline resistant percentages among *E. coli* in our study were lower (76.1%) than tetracycline-resistant *E. coli* in Vietnam (93.4%).

For the resistance to tetracycline, *S.* Albany in this study yielded a higher resistance (84.6%) than that of *S.* Albany from chicken meat and lettuce in Bangkok and Central Thailand in 2015 (60%, $n = 5$) [17], and from clinical patients, meats, cattle, chicken and pigs in Vietnam in 2004 (66.6%, $n = 3$) [18]. Our study showed 100% of *S.* Albany was resistant to sulfamethoxazole, the same as observed in the Vietnam in 2004 and Thailand in 2001–2006 (100%, $n = 3$ *vs* 100%, $n = 3$) [18,19]. Resistances to tetracycline and sulfamethoxazole of *S.* Corvallis from our study (79.0% and 76.7%) were lower than among *S.* Corvallis isolated from humans and non-human sources in Bangkok [19], showing respectively 82% and 94% of resistance to tetracyclines and sulfamethoxazole. During the Bangkok and Central Thailand study in 2015, two *S.* Kentucky strains were isolated from chicken, and only one strain was observed resistant to nalidixic acid [17], while in our study we isolated 69 *S.* Kentucky with 67 MDR strains highly resistant to tetracycline and nalidixic acid (both 97.1%), and 92.7%–94.2% to amoxicillin and sulfamethoxazole.

There was a high percentage of *E. coli* resistant to amoxicillin harboring $bla_{Tem,}$ for 62% in our study, a moderate percentage of 35% in *E. coli* positive by the $bla_{Tem\text{-}1b}$ presence isolated from canine urinary tracts were observed in Taiwan in 2015 [20] follows by two *S.* Albany with 8% positive isolates by the bla_{Tem}, and thirteen *S.* Kentucky amoxicillin-resistant (20%) contained 963 bp sequences similar to bla_{Tem} gene sequences.

Aminoglycoside nucleotidyl-transferases can confer resistance to gentamicin, tobramycin or streptomycin including *aad* and variant StrA/B among Gram-negative bacteria [21]. Thus, the *aadA,* S*trA*, and *aadA* + *StrA* genes have been found positive in *E coli* of the study, and both *aadA* and *StrA* genes were also observed positive among *S.* Corvallis and *S.* Kentucky.

The frequency distribution of the *sul* genes in Vietnam, 2007, in the three environments investigated, swine farms, shrimp ponds, and a city canal generally followed *sul1* > *sul2* > *sul3* [22], it was collaborated with the occurrences of the *sul* gene in our study for *S.* Albany and Kentucky, but it was not true for *E. coli* and *S.* Corvallis resistant to sulfamethoxazole (Table 3).

Quinolone resistances, in *E. coli* and *Salmonella* are mainly associated with mutations in the quinolone resistance determining region of the *GyrA* and *parC* genes [21], more than 75% of *E. coli*, and *Salmonella* serovars resistant to nalidixic acid were found to harbor the *gyrA* gene in our study.

In the present study, the tetracycline resistance coding efflux gene in *E. coli* strains was mainly mediated by 86% *Tet* (A) gene, 7% *Tet* (B) gene and 2% *Tet* (A) + *Tet* (B) gene, and these combinations were as well observed in *Enterobacteriaceae* isolated from integrated fish farms of South China [23], but for *S.* Albany, *S.* Corvallis, and *S.* Kentucky in this study, it was mediated only by the *Tet (A)* gene.

In conclusion, chicken food chains in Cambodia were be reservoirs of highly diverse and abundant antibiotic resistance genes. These resistance genes pose potential health risks to the public and animal husbandry in the country.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Support for this project was provided by the World Health Organization under AGISAR grant agreement 2012/246994-0 on 03 July 2012, and the Food and Agriculture Organization of the United Nation agreement LoA/RP/CMB/2011/AGNDC/PO280544 on 07 December 2011.

References

[1] Szmolka A, Nagy B. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. *Front Microbiol* 2013; **4**: 258.

[2] Qadri F, Svennerholm AM, Faruque AS, Sack RB. Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clin Microbiol Rev* 2005; **18**(3): 465-83.

[3] Allocati N, Masulli M, Alexeyev MF, Ilio CD. Escherichia coli in Europe: an overview. *Int J Environ Res Public Health* 2013; **10**(12): 6235-54.

[4] Van TTH, Nguyen HNK, Smooker PM, Coloe PJ. The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica*

isolated from producing -animals, retail meat and human in South-East Asia. *Int J Food Microbiol* 2012; **154**(3): 98-106.

[5] Yombi JC, Jonckheere S, Colin G, Van Gompel F, Bigare E, Belkhir L, et al. Clinical features and outcome of typhoid fever and invasive non-typhoidal salmonellosis in a tertiary hospital in Belgium: analysis and review of the literature. *Acta Clin Belg* 2013; **68**(2): 101-6.

[6] Bhan MK, Bahl R, Bhatnagar S. Typhoid and paratyphoid fever. *Lancet* 2005; **366**: 749-62.

[7] Crump JA, Mintz ED. Global trends in typhoid and paratyphoid Fever. *Clin Infect Dis* 2010; **50**(2): 241-6.

[8] Nguyen VT, Carrique-mas JJ, Ngo TH, Ho HM, Ha TT, Campbell JI, et al. Prevalence and risk factors for carriage of antimicrobial-resistant *Escherichia coli* on household and small-scale chicken farms in the Mekong Delta of Vietnam. *J Antimicrob Chemother* 2015; **70**(7): 2144-52.

[9] Kruy SL, Yith VT, Ping S, Khem P, Sarthou JL. Prevalence, numbers and antimicrobial susceptibilities of *Salmonella* serovars and *Campylobacter* spp. in retail poultry in Phnom Penh, Cambodia. *J Vet Med Sci* 2011; **73**(3): 325-9.

[10] Vlieghe ER, Phe T, De Smet B, Veng CH, Kham C, Bertrand S, et al. Azithromycin and ciprofloxacin resistance in *Salmonella* bloodstream infections in Cambodian adults. *PLoS Negl Trop Dis* 2012; **6**(12). e1933.

[11] Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* 2010; **74**(3): 417-33.

[12] Olesen I, Hasman H, Aarestrup FM. Prevalence of β-lactamases among ampicillin-resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. *Microb Drug Resist* 2004; **10**(4): 334-40.

[13] Aarestrup FM, Lertworapreecha M, Evans MC, Bangtrakulnonth A, Chalermchaikit T, Hendriksen RS, et al. Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar Weltevreden from different countries. *J Antimicrob Chemother* 2003; **52**(4): 715-8.

[14] Gebreyes WA, Thakur S. Multidrug-resistant of *Salmonella enterica* serovar Muenchen from pig and human and potential interserovar transfer of antimicrobial resistance. *Antimicrob Agents Chemother* 2005; **49**(2): 503-11.

[15] Levings RS, Partridge SR, Lightfoot D, Hall MR, Djordjevic SP. New integron-associated gene cassette encoding a 3-N-amino-glycoside acetyltransferase. *Antimicrob Agents Chemother* 2005; **49**(3): 1238-41.

[16] Brown JC, Thomson CJ, Amyes SG. Mutations of the gyrA gene of clinical isolates of *Salmonella* Typhimurium and three other *Salmonella* species leading to decreased susceptibilities to 4-quinolone drugs. *J Antimicrob Chemother* 1996; **37**(2): 351-6.

[17] Niyomdecha N, Mungkornkaew N, Samosornsuk W. Serotypes and antimicrobial resistance of *Salmonella enterica* isolated from pork, chicken meat and lettuce, Bangkok and Central Thailand. *Southeast Asian J Trop Med Public Health* 2016; **47**(1): 31-9.

[18] An TTV, van Duijkeren E, Gaastra W, Fluit AC. Antimicrobial resistance, class 1 integrons, and genomic island in *Salmonella* isolates from Vietnam. *Plos One* 2010; **5**(2): e9440.

[19] Sirichote P, Bangtrakulnonth A, Tianmanee AU, Oulai A, Chittaphithakchai WK, Hendriksen RS. Serotypes and antimicrobial resistance of *Salmonella enterica* spp. in Central Thailand, 2001–2006. *S A J Trop Med Pub Health* 2010; **41**(6): 1405-15.

[20] Shao KC, Dan YL, Hen W, Hung CK. Antimicrobial resistance of *Escherichia* isolates from canine urinary tract infections. *J Vet Med Sci* 2015; **77**(1): 59-65.

[21] Frye GJ, Jackson CR. Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica, Escherichia coli*, and *Enterococcus* spp. isolated from US food animals. *Front Microbiol* 2013; **4**: 135.

[22] Phuong HPT, Nonaka L, Hung VP, Suzuki S. Detection of the *sul1*, *sul2*, and *sul3* genes in sulfonamide-resistant bacteria from waste water and shrimp ponds of North Vietnam. *Sci Total Environ* 2008; **405**(1–3): 377-84.

[23] Su HC, Ying GG, Tao R, Zhang RQ, Fogarty LR, Kolpin DW. Occurrence of antibiotic resistance and characterization of resistance genes and integrons in Enterobacteriaceae isolated from integrated fish farms in south China. *J Environ Monit* 2011; **13**(11): 3229-36.