MULTI-DRUG RESISTANCE CHARACTERISTICS OF *E. COLI* ISOLATED FROM WATER SOURCES IN CHICKEN FARMS, KHON KAEN, THAILAND

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Abstract. The study investigated antimicrobial resistance patterns and presence of resistance genes to nine drugs (five drug classes) of *Escherichia coli* isolated from water sources (water tank, drinking water, environmental/waste water, and evaporation pond) at ten chick rearing farms in the vicinity of Khon Kaen city, Khon Kaen Province, Thailand during 2011-2012. Of 52 *E. coli* strains isolated, 61% were multi-drug resistant (≥3 classes of drugs) and 4-79% of strains were sensitive to all nine drugs tested. Highest (86%) and lowest (21%) resistance prevalence was against cefotaxime and cefoxitin, respectively. The frequency of β-lactamase-producing gene, *bla*TEM-1, carried by the *E. coli* strains reflected the genotypic characteristics. These alarming findings are indicative of an urgent need for stricter enforcement of good hygienic practices in the poultry industry.

Keywords: *Escherichia coli,* chicken farm, multi-drug resistance, water source, Thailand

INTRODUCTION

Escherichia coli is a gram-negative bacterium of the family Enterobacteriaceae. It is a normal flora of intestinal tracts of humans and animals, including birds (Singleton and Sainsburg, 1981; St-Pierre *et al,* 2009). Most strains of *E. coli* are harmless (Trongjit *et al,* 2016). However, it is one of the opportunistic pathogens responsible for a number of disease conditions in chicken such as enteritis (Nhung *et al,* 2016). Pathogenic *E. coli* released from fecal content of infected humans and animals can be found in various water resources, such as river, stream, creek, and well. Waste can enter the water system through many different ways, *viz.* sewage overflow, improper operating sewage system, and polluted storm water and agricultural runoffs (He *et al,* 2007). Wells are more vulnerable to such contamination after flooding, in particular if the wells are shallow, have been dug or bored, or have been submerged by floodwater for long periods of time (He *et al,* 2007).

Physical, chemical, and microbiological qualities of drinking water are of fundamental importance in chicken

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industry (Jafari *et al,* 2006). The role of water in spreading communicable diseases is highly likely if it comes from a number of different sources. Water contaminated with fecal coliform from sick birds, animals, and humans severely affects the performance of birds (Desmarais *et al,* 2002). In addition to pathogenic *E. coli*, *Salmonella* and *Campylobacter* spp constitute the three main chicken pathogens responsible for water contamination (Jafari *et al,* 2006; FAO, 2007).

Antimicrobial usage in animal production and in human clinical treatment are similar (FAO, 2007; Apisamthanarak *et al,* 2009) and consequently, emergence of antimicrobial resistance (AMR) poses a critical threat to both human and animal welfare. Thailand is one of the Southeast Asia economic community considered to be a focal point for AMR in bacteria from various origins (Coker *et al,* 2011; Von Wintersdorff *et al,* 2014; WHO, 2014). A number of countries in this region, namely, Indonesia, Thailand and Vietnam, have considerably developed over many decades their aquaculture and, particularly in the case of Thailand, poultry production, mostly for export (FAO, 2009; FAO, 2016). This creates a further risk of dissemination of AMR organisms to consumers worldwide (Nhung *et al,* 2016).

Hence, the current study was conducted to determine the antibiogram profiles of *E. coli* isolated from four types of waters at 10 broiler chicken farms around the city of Khon Kaen, northeastern Thailand. Both multi-drug resistance phenotypes and genotypes of *E. coli* isolates were determined. This would provide pertinent and essential information to reduce the prevalence of multi-drug resistant *E. coli* isolated from animals and from humans within the surrounding region.

MATERIALS AND METHODS

Study locations

Ten broiler chicken farms located in Wang Noi Dictrict, Khon Kaen Province, northeastern Thailand, which produce chickens for export were enrolled during 2011-2012 in an "all in/all out" scheme in which 1-day old chicks are reared and slaughtered all at once. The farms housed approximately 10,000-20,000 birds depending on size of the housing facility.

Water samples

For each farm four types of water samples were collected: (i) main water tank, (ii) drinking water line (three lines per farm), (iii) environmental/waste water sample, and (iv) evaporation pond. Water samples were tested twice during the summer (March-June, 2011), rainy (July-October, 2011), and winter (November, 2011-February, 2012) seasons when the chickens were aged between 1-15 days (first round) and 16-35 days (second round). Water samples (2,500 ml aliquots) were collected aseptically into sterile plastic bags and transported on ice to the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand within 4 hours. A 2-ml of autoclaved 1.0% sodium thiosulphate solution (Amersco, Fountain Parkway, Solon, OH) was aseptically added into each sterile plastic bag as a chlorine neutralizer for main tank and drinking water samples (Savill *et al,* 2001; Shaikh *et al,* 2015).

Isolation and identification of *E. coli* isolates

E. coli was isolated from the samples based on typical characteristics (blue colonies associated with gas bubbles) on 3M Petri film (OMA #991.14; 3M Food Safety; 3M Center, St Paul, MN) following filtration. A PCR procedure was employed

for confirmation as *E. coli* using specific primer pair targeting 16S rDNA, ECO-1 (5′GACCTCGGTTTAGTTCACAGA3′) and ECO-2 (5′CACACGCTGACGCTGA CCA3′) (Wang *et al,* 1996). Direct colony PCR was performed. Reaction mixture (25 µl) consisted of bacterial cells as source of DNA template, Jumpstart RED Taq Ready mix (Merck, Darmstadt, Germany), specific primer pairs (Thermo Scientific, Singapore), and PCR grade water (Thermo Scientific). Thermocycling was performed in an EsCo Swift MiniPro thermal cycler (Esco Micro, SWT-MIP-0.2-2, Singapore) as follows: 95°C for 3 minutes; 35 cycles of 95°C for 30 seconds, 58°C for 45 seconds and 72°C for 60 seconds; with a final step of 72°C for 7 minutes. Amplicon (585 bp) was analyzed by 1.5% agarose gel-electrophoresis, stained with ethidium bromide (Merck, Darmstadt, Germany) and recorded using a gel documentation system (Syngene®, Cambridge, UK).

Antibiogram determination

E. coli isolates were cultured on Nutrient agar plate (HiMedia, Mumbai, India), grown for 18 hours, then harvested and suspended in 0.1% normal saline solution, adjusted to 0.5% McFarland unit ($1.5x10^8$ CFU/ml), and (0.1 ml aliquots) spread onto the surface of Muller Hinton agar (MHA) (Oxoid, Basingstoke, Hamshire, UK) plates to produce bacterial lawns. Kirby-Bauer disk diffusion method was employed for determination of antibiograms (CLSI, 2017). *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were employed as controls. Five groups of drugs (Oxoid) were evaluated, namely, aminoglycoside [10 µg of gentamicin (GEN) and 10 µg of tobramycin (TOB)], cephalosporin [30 µg of cefotaxime (CTX), 30 µg of cefoxitin (FOX) and 30 µg of cephazolin (KZ)], quinolone [5 µg of ciprofloxacin (CIP) and 5 µg of ofloxacin (OFX)], and tetracycline [30 µg of doxycycline (DO)], as well as 5 µg of anti-folate trimethoprim (W). Resistance to ≥3 groups of drugs is considered as multidrug resistant (CLSI, 2017).

Detection of *E. coli* antimicrobial genes

Primer pairs targeting *E. coli aac(3)-Ia, aph(3′)-IIa,* ^{bla}TEM-1, *dhfr*I, *sul*I, *sul*II, *tet*A, and *tet*B were as previously published (Zhao *et al,* 2001; Chen *et al,* 2004; Muhammad *et al,* 2009; FAO, 2016). In brief, PCR was conducted in a 25-µl mixture containing bacterial cells as source of DNA template, Jumpstart RED Taq Ready mix (Merck), specific primer pairs (Thermo Scientific), and PCR grade water (Thermo Scientific). Thermocycling was performed as described above using annealing temperature at 54°C for *aac(3′)-Ia, aph(3′)-IIa, dhfr*I, *sul*I, *sul*II, and *tet*B, 55°C for bla_{TEM-1}, and 58°C for *tet*A (Lé Vesque *et al,* 1995; Zhao *et al,* 2001; Chen *et al,* 2004; Johnson *et al,* 2007; Ma *et al,* 2007; Overdevest *et al,* 2011; FAO, 2016; Trongjit *et al,* 2016). Amplicons [436 bp *(aac(3)-Ia)*, 519 bp *(aph(3′)-IIa)*, 643 bp *(*^{bla}TEM-1*)*, 220 bp *(dhfr*I*)*, 331 bp *(sul*I*)*, 435 bp *(sul*II*)*, 831 bp *(tet*A*)*, and 723 bp *(tet*B*)*] were analyzed and recorded as described above.

Statistical analysis

Descriptive statistics were used to show means and percentages of data employing SPSS statistical package version 20.0 (IBM, Armonk, NY). Chi-square test was conducted for testing of effect of

season on *E. coli* detection rate. A *p*-value <0.05 is considered significant.

RESULTS

In total, 350 water samples were tested in the study as, on some occasions, water samples could not be collected because the chicken farm owners were absent, and in some farms all chickens had been sold before the visit. Among 52 *E. coli* isolates available for PCR confirmation, which varied from 4-19% per farm (Table 1), 24 were from drinking water sources, 17 from evaporation ponds, 9 from environmental/waste water sources, and 2 from main water tanks. There are no significant difference among the three seasons (summer, rainy and winter) on the detection rate of *E. coli.*

The *E. coli* strains were most sensitive (79%) to FOX (2nd generation cephalo-

Table 1
PCR-confirmed *E. coli* isolates from water samples of ten chicken farms, Wang Noi District, Khon Kaen Province, Thailand during 2011-2012.

Farm no.	Number (%) (*n*=52)
F1	7 (13)
F2	2 (4)
F3	6 (11)
F4	7 (13)
F5	10 (19)
F6	4 (8)
F7	4 (8)
F8	5 (10)
F9	5 (10)
F10	2 (4)

Table 2
Antibiograms of PCR-confirmed *Escherichia coli* strains isolated from water samples of ten chicken farms, Wang Noi District, Khon Kaen Province, Thailand during 2011-2012.

Antimicrobial group	Antimicrobial	Dose (μg)	Percent *E. coli* susceptibility (*n*=52)		
			S	I	R
Aminoglycoside	Gentamicin	10	44	23	33
	Tobramycin	10	23	13	64
Cephalosporins	Cephazolin	30	4	27	69
	Cefoxitin	30	79	11	10
	Cefotaxime	30	13	0	87
Quinolone	Ciprofloxacin	5	14	13	73
	Ofloxacin	5	32	8	60
Anti-folate	Trimethoprim	5	27	31	42
Tetracycline	Doxycycline	30	50	36	14

I, intermediate; R, resistance; S, susceptible [according to CLSI (2017) guidelines].

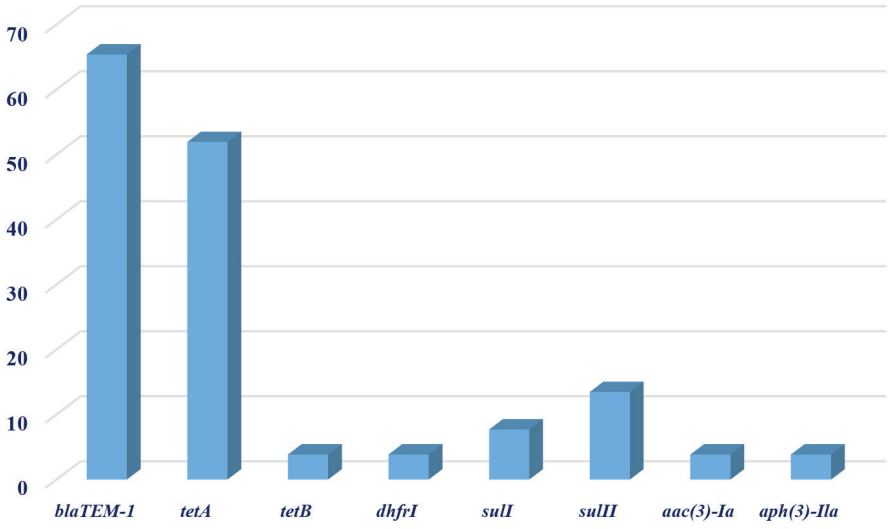

Fig 1 - Prevalence (%) of genes encoding antimicrobial resistance found in *Escherichia coli* isolated from water samples of ten chicken farms, Wang Noi District, Khon Kaen Province, Thailand during 2011-2012. *E coli* antimicrobial resistance genes were detected by PCR amplification using gene-specific primers.

sporin) followed by DO (50%) (Table 2). The highest resistance (87% of the strains) was against CTX (3rd generation cephalosporin) followed by CIP (73%) and KZ (1st generation cephalosporin) (69%). Multidrug resistance was present in 61% of the strains (Table 3). Strikingly, one *E. coli* strain from a drinking water sample collected from Farm no. 1 at the first round (1-15 day-old chickens) in the rainy season was resistant to nine antimicrobials from all five groups of antibiotics (CTX, CIP, DO, FOX, GEN, KZ, OFX, TOB, and W). Frequencies of the presence of antimicrobial genes, as expected, mirrored those of the corresponding drugs: 65% for *bla*TEM-1, 52% *tet*A and 13% *sul*II (Fig 1).

DISCUSSION

This study demonstrates *E. coli* isolates from different water sources (main water tank, drinking water, environmental/waste water, and evaporation pond) of ten poultry farms located near a city in northeast Thailand were resistant to five different groups of antibiotics. The nine drugs selected for assay consist mainly those in the treatment of gram-negative bacilli infections worldwide. Some of the antibiotics are used in chicken production process as performance enhancers (Oguttu *et al*, 2008). It is well acknowledged that aminoglycosides, cephalosporin, fluoroquinolones, and trimethoprim are drug groups that have major uses for enhancing poultry production as well as for therapeutic treatment (CLSI, 2017). Some 85% of the *E. coli* isolates were resistant to the 3rd generation cephalosporin CTX. It is known that the rates of resistance to 3rd generation cephalosporins are increasing (Baron *et al*, 2014) and this problem needs closer attention as it directly impacts human health, given that *E. coli* is the most common bacterium causing invasive disease in man (Cogliani *et al*, 2011; de Kraker

Table 3
Multi-drug resistance antibiograms of PCR-confirmed *Escherichia coli* strains isolated from water samples of ten chicken farms, Wang Noi District, Khon Kaen Province, Thailand during 2011-2012.

Number of antimicrobials resistance	Resistance pattern	Number of strains (%) (n=52)[a]
1R	FOX (1 drug group)	1(2)
	W (1 drug group)	1(2)
	CTX (1 drug group)	2(4)
	Subtotal	4(8)
2R	KZ-CTX (1 drug group)	1(2)
	CTX-TOB (2 drug groups)	1(2)
	Subtotal	2(4)
3R	CIP-W-OFX (2 drug groups)	2(4)
	KZ-CTX-TOB (2 drug groups)	3(6)
	KZ-CTX-CIP (2 drug groups)	1(2)
	CTX-TOB-CIP (3 drug groups)	1(2)
	CIP-W-OFX (2 drug groups)	2(4)
	CTX-TOB-GEN (2 drug groups)	1(2)
	Subtotal	10(20)
4R	KZ-CTX-CIP-OFX (2 drug groups)	4(8)
	CTX-TOB-CIP-OFX (3 drug groups)	1(2)
	KZ-CTX-TOB-CIP (3 drug groups)	1(2)
	CTX-CIP-W-OFX (3 drug groups)	1(2)
	KZ-CTX-TOB-W (3 drug groups)	2(4)
	KZ-CTX-TOB-GEN (2 drug groups)	1(2)
	Subtotal	10(20)
5R	KZ-CTX-TOB-CIP-OFX (3 drug groups)	4(8)
	KZ-CTX-TOB-CIP-GEN (3 drug groups)	1(2)
	KZ-CTX-CIP-W-OFX (3 drug groups)	2(4)
	KZ-CTX-TOB-CIP-W (4 drug groups)	1(2)
	Subtotal	8(16)
6R	KZ-CTX-TOB-CIP-GEN-OFX (3 drug groups)	3(6)
	KZ-CTX-CIP-GEN-OFX-DO (4 drug groups)	2(4)
	KZ-CTX-TOB-CIP-W-OFX (4 drug groups)	1(2)
	KZ-CTX-TOB-CIP-GEN-W (4 drug groups)	1(2)
	KZ-CTX-CIP-GEN-W-OFX (4 drug groups)	1(2)
	CTX-TOB-CIP-W-OFX-DO (5 drug groups)	1(2)
	CTX-TOB-CIP-GEN-W-OFX (4 drug groups)	1(2)
	Subtotal	10(20)
7R	KZ-CTX-TOB-CIP-GEN-OFX-DO (4 drug groups)	2(4)
	KZ-CTX-TOB-CIP-FOX-OFX-DO (4 drug groups)	1(2)
	KZ-CTX-TOB-CIP-GEN-W-OFX (4 drug groups)	3(6)
	KZ-CTX-TOB-CIP-W-FOX-OFX (4 drug groups)	1(2)
	Subtotal	7(14)
9R	KZ-CTX-TOB-CIP-GEN-W-FOX-OFX-DO (5 drug groups)	1(2)
	Multi-drug resistance strain[b]	32(61)

[a]>100 due to rounding-up of numbers. [b]Resistant to ≥3 groups of drugs (CLSI, 2017). nR = resistance to n drugs. CIP, ciprofloxacin; CTX, cefotaxime; DO, doxycycline; FOX, cefoxitin; GEN, gentamicin; KZ, cephazolin; OFX, ofloxacin; TOB, tobramycin; W, trimethoprim.

et al, 2011; ECDPC, 2011; Collignon *et al*, 2013). In addition, similar high resistance rates were observed for KZ (a 1st generation cephalosporin) and CIP (a quinolone). Although resistance to FOX and DO were relatively low in our study, Oguttu *et al* (2008) in South Africa reported a much higher resistance rate (98.2%) against DO. A large proportion of antimicrobial resistant bacteria in human infections is derived from food animals (Johnson *et al*, 2007; Jakobsen *et al*, 2010; Overdevest *et al*, 2011; Vieira *et al*, 2011; Collignon *et al*, 2013). It is noteworthy that only one *E. coli* strain collected in the current study was susceptible to all nine antibiotics (in the range of 4-79%).

From previous studies (Le'Vesque *et al*, 1995; Chen *et al*, 2004; Ma *et al*, 2007; Germeau-Tsodikova and Labby, 2016) and from the phenotypic and genotypic assessments of *E. coli* isolates in the current study, it is most likely *aph(3')-IIa* was responsible for GEN resistance and *aac* (3')-*Ia* for TOB resistance in the tested aminoglycoside group. However, further study is warranted.

A study in Sa Keao Province, eastern Thailand showed a similar finding to the current study in that *bla*TEM-1 is the most prevalent gene found in chicken and pig carcasses (Trongjit *et al*, 2016).

In summary, the study reveals a high prevalence of multi-drug resistance among *E. coli* strains present in various sources of water, ranging from drinking water to evaporation pond, in chicken farms located in the vicinity of a major city in northeastern Thailand. Resistance was associated with antimicrobial resistance genes carried by the isolated *E. coli* and the bacteria were resistant to at least one of nine antibiotics commonly prescribed in the treatment of gram-negative pathogenic bacteria. Thus, crucial and urgent precautionary steps need to be employed to stop the rise of antimicrobial resistant *E. coli* (and other pathogenic bacteria) in poultry farms through implementation of robust hygienic husbandry practices especially in the supply of drinking water. Future studies should focus on the impact of coliform-contaminated water from the chicken farms on the surrounding human health and environment.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Apisamthanarak A, Buppunharun W, Tiengnm S, Sawanpanyalert P, Aswapokee N. An overview of antimicrobial susceptibility patterns for gram-negative bacteria from the National Antimicrobial Resistance Surveillance Thailand (NARST) program from 2000 to 2005. *J Med Assoc Thai* 2009; 92 (Suppl 4): S91-4.

Baron S, Jouy E, Larvor E, Eono F, Bougeard S, Kempf I. Impact of third-generation -cephalosporin administration in hatcheries on fecal *Escherichia coli* antimicrobial resistance in broilers and layers. *Antimicrob Agents Chemother* 2014; 58: 5428-34.

Chen S, Zhao S, White DG, *et al.* Characterization of multiple-antimicrobial-resistant Salmonella serovars isotated from retail meats. *Appl Environ Microbiol* 2004; 70 : 1-7.

Clinical and Laboratory Standards Institute. (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty Second Informational Supplement. CLSI document M100-27. Wayne: CLSI, 2017.

Cogliani C, Goossens H, Greko C. Restricting antimicrobial use in food animals : lessons from Europe. *Microbe* 2011; 6 : 274-9.

Coker RJ, Hunter BM, Rudge JW, Liverani M, Hanvoravongchai P. Emerging infectious diseases in Southeast Asia: regional challenges to control. *Lancet* 2011; 377: 599-9.

Collignon P, Aarestrup FM, Irwin R, Mc Ewen S. Human deaths and third-generation cephalosporin use in poultry, Europe. *Emerg Infect Dis* 2013; 19: 1339-40.

de Kraker ME, Davey PG, Grundmann H. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. *PLOS Med* 2011; 8: e1001104.

Desmarais TR, Solo-Gabriele HM, Palmer CJ. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl Environ Microbiol* 2002; 68: 1165-72.

European Centre for Disease Prevention and Control (ECDPC). Antimicrobial resistance surveillance in Europe 2010. Annual report of the European Antimicrobial Resistance Surveillance Network (EARSNet). Stockholm: ECDPC, 2011.

Food and Agriculture Organization (FAO). Joint FAO/WHO/OIE expert meeting on critically important antimicrobials. Rome: FAO, 2007.

Food and Agriculture Organization (FAO). Analysis of aquaculture development in Southeast Asia: a policy perspective. Rome: FAO, 2009. [Cited 2017 Nov 1]. Available from: http://www.fao.org/docrep/012/i0950e/i0950e00.pdf

Food and Agriculture Organization (FAO). Livestock industrialization project: phase II-policy, technical, and environmental determinants and implications of the scalding-up of swine, broiler, layer and milk production in Thailand. Rome: FAO, 2016. [Cited 2017 Nov 1]. Available from: http://www.fao.org/docrep/012/i0950e/i0950e00.pdf

Garmeau-Tsodikova S, Labby KJ. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. *Medchemcomm* 2016; 7: 11-27.

He L-M, Lu J, Shi W. Variability of fecal indicator bacteria in flowing and ponded waters in southern California: implications for bacterial TMDL development and implementation. *Water Res* 2007; 41: 3132-40.

Jafari R A, Fazlara A, Govahi M. An investigation into *Salmonella* and fecal coliform contamination of drinking water in broiler farms in Iran. *Int J Poult Sci* 2006; 5: 491-3.

Jakobsen L, Spangholm D J, Pedersen K, *et al.* Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in *Escherichia coli* isolates from community-dwelling humans and UTI patients. *Int J Food Microbiol* 2010; 142: 264-72.

Johnson JR, Sannes MR, Croy C, *et al.* Antimicrobial drug–resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002-2004. *Emerg Infect Dis* 2007; 13: 838-46.

Le´Vesque C, Piche´ L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Chemother* 1995; 39: 185-91.

Ma M, Wang H, Yu Y, Zhang D, Liu S. Detection of antimicrobial resistance genes of pathogenic *Salmonella* from swine with DNA microarray. *J Vet Diagn Invest* 2007; 19: 161-7.

Muhammad AA, Saidul A, Hassan SMR,

Momena S. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *Int J Food Safety* 2009; 11: 19-23.

Nhung NT, Cuong NV, Thwaites G, Carrique-Mas J. Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. *Antibiotics* 2016; 5: 37. doi: 10.3390/antibiotics5040037.

Oguttu JW, Veary CM, Picard JA. Antimicrobial drug resistance of *Escherichia coli* isolated from poultry abattoir workers at risk and broilers on antimicrobials. *J South Vet Assoc* 2008; 79: 161-6.

Overdevest I, Willemsen I, Rijnsburger M, *et al*. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. *Emerg Infect Dis* 2011; 17: 1216-22.

Savill MG, Hudson JA, Ball A. Enumeration of *Campylobacter* in New Zealand recreational and drinking waters. *J Appl Microbiol* 2001; 91: 38-46.

Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. *Saudi J Biol Sci* 2015; 22: 90-101.

Singleton P, Sainsburg D. Singleton dictionary of microbiology. International ed. New York: John Willey and Sons, 1981.

St-Pierre K, Levesque S, Frost E, Carriers N, Arbeit RD, Michaud S. Thermotolerant coliforms are not a good surrogate for *Campylobacter* spp in environmental water. *Appl Environ Microbiol* 2009; 75: 6736-44.

Trongjit S, Angkittitrakul S, Chuanchuen R. Occurrence and molecular characteristics of antimicrobial resistance of *Escherichia coli* from broilers, pigs, and meat products in Thailand and Cambodia provinces. *Microbiol Immunol* 2016; 60: 575-85.

Vieira AR, Collignon P, Aarestrup FM, *et al*. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: an ecological study. *Foodborne Pathog Dis* 2011; 8: 1295-301.

Von Wintersdorff CJ, Penders J, Stobberingh EE. High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. *Emerg Infect Dis* 2014; 20: 649-57.

Wang R-F, Cao W-W, Cerniglia ACE. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Appl Environ Microbiol* 1996; 62: 1242-7.

World Health Organigation (WHO). Antimicrobial resistance: global report on surveillance. Geneva: WHO, 2014. [Cited 2017 Nov 1]. Available from: http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1

Zhao S, White DG, Ge B, *et al*, Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. *Appl Environ Microbiol* 2001; 67: 1558-64.