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Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes

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ABSTRACT

This study was conducted to examine a current baseline profile of antimicrobial resistance and virulence of *Escherichia coli* isolated from foods commonly sold in the market place in Vietnam. *E. coli* were isolated from 180 samples of raw meat, poultry and shellfish and also isolated from 43 chicken faeces samples. Ninety-nine *E. coli* isolates recovered from all sources were selected for the investigation of their susceptibility to 15 antimicrobial agents by the disk diffusion method. Eighty-four percent of the isolates were resistant to one or more antibiotics, and multi-resistance, defined as resistance to at least 3 different classes of antibiotics, was detected in all sources. The rates of multi-resistance were up to 89.5% in chicken, 95% in chicken faeces and 75% in pork isolates. Resistance was most frequently observed to tetracycline (77.8%), sulfafurazole (60.6%), ampicillin (50.5%), amoxicillin (50.5%), trimethoprim (51.5%), chloramphenicol (43.4%), streptomycin (39.4%), nalidixic acid (34.3%) and gentamicin (24.2%). In addition, the isolates also displayed resistance to fluoroquinolones (ciprofloxacin 16.2%, norfloxacin 17.2%, and enrofloxacin 21.2%), with chicken isolates showing the highest rates of resistance to these antibiotics (52.6–63.2%). Thirty-eight multi-resistant isolates were selected for further the examination of antibiotic resistance genes and were also evaluated for virulence gene profiles by multiplex and uniplex polymerase chain reaction. The beta-lactam TEM gene and tetracycline resistance *tetA*, *tetB* genes were frequently detected in the tested isolates (84.2% and 89.5% respectively). Genes which are responsible for resistance to streptomycin (*aadA*) (68.4%), chloramphenicol (*cmlA*) (42.1%), sulfonamides (*sulI*) (39.5%), trimethoprim (*dhfrV*) (26.3%) and kanamycin (*aphA-1*) (23.7%) were also widely distributed. Plasmid-mediated *ampC* genes were detected in *E. coli* isolates from chicken and pork. The isolates were tested for the presence of 58 virulence genes for adhesins, toxins, capsule synthesis, siderophores, invasins and others from different *E. coli* pathotypes. All of the tested isolates contained at least one virulence gene and there were 16 genes detected. Virulence genes detected were *fimH* (92.1%), *bmaE* (84.2%), TSPE4.C2 (42.1%), *aidA* AIDA-I (*orfB*) (31.6%), *east1* (26.3%), *traT* (23.7%), and others including *fyuA*, *iutA*, *chuA*, *yjaA*, *iss*, *iroN*$_{E.\ coli}$, *ibeA*, *aah* (*orfA*), *iha* and *papG* allele III (10.5–2.6%). Typical toxin genes produced by enterohemorrhagic and enterotoxigenic *E. coli* pathotypes (a heat-stable toxin (ST), heat-labile toxin (LT) and Shiga toxin *stx1*, *stx2*) were not detected in any of these 38 isolates. The study has revealed that *E. coli* in raw foods is a significant reservoir of resistance and virulence genes.

1. Introduction

Foodborne diseases due primarily to bacteria, viruses, parasites, and chemicals are an important cause of morbidity and mortality worldwide. Bacteria are probably the most common cause of illness (Mead et al., 1999; Lindqvist et al., 2000; Adak et al., 2002; Su et al., 2005; Lynch et al., 2006). Foodborne bacteria infections with diarrhoea symptoms are usually self limiting. However, systemic infection and ensuing death can occur, particularly in vulnerable groups with diminished immunity such as the elderly, infants and young children (Mead et al., 1999; Meng and Doyle, 2002; Kennedy et al., 2004). Treatment options for foodborne gastroenteritis may require fluid and electrolyte replacement and antibiotics are usually prescribed in severe cases (Nataro and Kaper, 1998; Hohmann, 2001; Huang et al., 2006).

Foods contaminated with antibiotic resistant bacteria could be a major threat to public health as there is the distinct possibility that genes encoding antibiotic resistance determinants that are carried on mobile genetic elements may be transferred to other bacteria of human clinical significance. *E. coli* is a candidate vehicle for such transfers because of its diversity and also because it survives as

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common flora in the gastrointestinal tracts of both humans and animals. They are sensitive to selection pressure exerted by antibiotic usage and carry genetic mobile elements to achieve such transmission (Van den Bogaard and Stobberingh, 2000). Although the carriage of antibiotic resistance genes is not confined to commensal *E. coli* in the face of antibiotic selection, the capacity to threaten human consumers is significantly enhanced if foodborne strains carried virulence genes that qualified them as potential human pathogens (Orskov and Orskov, 1992; Schroeder et al., 2004).

Diarrhoeagenic *E. coli* strains are categorised into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O:H serotypes (Meng et al., 2001). The six main categories include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC or STEC), diffuse-adhering *E. coli* (DAEC) (Nataro and Kaper, 1998). There are regional differences in the prevalence of the different diarrhoeagenic *E. coli* categories (Albert et al., 1995; Ratchtrachenchai et al., 2004; Nguyen et al., 2006). Amongst all of these different pathotypes, faecal STEC contamination of raw meats and poultry represent the most commonly reported food safety problems (Guth et al., 2003; Conedera et al., 2004; Carney et al., 2006; Samadpour et al., 2006). In contrast to diarrhoeagenic *E. coli*, extra-intestinal pathogenic *E. coli* (ExPEC) strains are incapable of causing enteric disease but responsible for most extra-intestinal infections (Kuhnert et al., 2000; Russo and Johnson, 2000; Johnson and Russo, 2002; Bekal et al., 2003).

Like in many other developing countries, raw food hygiene and antimicrobial resistance epidemiology is at its infancy in Vietnam. In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of foodborne microbes harbouring an array of resistance genes. This study was conducted to address some of these issues and to provide a current baseline profile of antimicrobial resistance and virulence of *E. coli* isolated from foods commonly sold in the market place in Vietnam. The strategy was to compare *E. coli* susceptibility to 15 antibiotics and the prevalence of corresponding genes encoding these resistances. The pathogenic potential of *E. coli* isolates based on their virulence gene profiles was also determined.

2. Materials and methods

2.1. E. coli isolation and identification

One hundred and eighty samples of meat comprising beef (n=50), chicken/poultry (n=30), pork (n=50) and shellfish (n=50) were purchased from various markets and supermarkets around Ho Chi Minh City between February and June 2004 for the isolation and identification of *E. coli*. Forty-three samples from chicken faeces were also collected from two chicken farms, chickens less than 1 month old. The procedures for isolation of *E. coli* were based on the Nordic Committee on Food Analysis method (NMKL, 1996).

2.2. Antibiotic susceptibility tests

Ninety-nine *E. coli* isolates from different sources (pork (n=20), beef (n=20), chicken (n=19), chicken faeces (n=20), shellfish (n=20)) were randomly selected from the *E. coli* collection for antibiotic susceptibility test to 15 antibiotics by the disk diffusion method on Mueller-Hinton agar plates, only one *E. coli* isolate was selected from one food sample. The standard procedure of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (NCCLS, 2004) were strictly followed throughout the testing procedure. Quality control strains *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were included in each run. The concentration of the discs (Oxoid, Australia) and abbreviation of antimicrobial agents which were used throughout this paper are: ampicillin (AMP) 10 μg, amoxicillin (AMX) 10 μg, amoxicillin/clavulanic acid (AMC) 30 μg, cephalothin (CEF) 30 μg, chloramphenicol (CHL) 30 μg, ciprofloxacin (CIP) 5 μg, enrofloxacin (ENR) 5 μg, tetracycline (TET) 30 μg, gentamicin (GEN) 10 μg, kanamycin (KAN) 30 μg, nalidixic acid (NAL) 30 μg, norfloxacin (NOR) 10 μg, sulphafurazole (SUL) 300 μg, streptomycin (STR) 10 μg, and trimethoprim (TMP) 5 μg. The isolates were classified as susceptible, intermediate, and resistant according to the zone diameter interpretative standards recommendations by CLSI (2005) and recorded as susceptible, intermediate, or resistant to each antibiotic tested.

2.3. Detection of antibiotic resistance and virulence genes

Thirty-eight multi-resistant isolates, which showed the highest degree of resistance among the collection, were examined for antibiotic resistance and virulence genes. These included *E. coli* isolates from chicken (n=14), beef (n=4), pork (n=8), shellfish (n=5), and chicken faeces (n=7). Nineteen of the isolates contained known class 1 resistance integrons (Van et al., 2007).

Twenty-two antibiotic resistance genes (ARGs) were screened by PCR using a combination of 3 multiplex and 3 uniplex assays. Sets 1 to 3 were designed to detect *sulI*, SHV, *cat1*, *dhfrV*, *floR*, *aadA*, OXA; TEM, *cmlA*, CITM, *ereA*, *dhfrI*, *aac(3)-I*); and *aphA-1*, MOXM, DHAM, EBCM, *aac(3)-IV*, FOXM genes respectively. The tetracycline resistance genes (*tetA*, *tetB* and *tetC*) were amplified individually (Table 1). A positive and a negative control for each PCR were included. The identity of all 22 ARG amplicons have been previously confirmed by sequencing (Wu, 2006). PCR reactions for multiplex sets 1–3 were performed in a total volume of 25 μl containing 2 μl of Chelex (BioRad) extracted DNA with final concentrations of 4 mM $MgCl_2$, 10 μM of each dNTP (Bioline), 5 μl of each primer pool and 1 U of Hotstart Taq (Qiagen). The uniplex (primer sets 4–6) PCR conditions were performed in a total volume of 50 μl containing 2 μl of Chelex (BioRad) extracted DNA with final concentration of 1.5 mM $MgCl_2$, 2.5 μM of each dNTP (Bioline), 0.5 μl of each primer pair and 1 U of Taq polymerase (Bioline). PCR amplification was conducted in Palmcyclers (Corbett Research) with the following conditions for multiplex sets 1–3: initial denaturation at 95 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min and final cycle of amplification at 72 °C for 10 min. The uniplex PCR amplification conditions consisted of initial denaturation at 94 °C for 5 min, with 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 1 min and final cycle of amplification at 72 °C for 10 min. Amplicons were visualized by electrophoresis at 80 V, 500 mA for 2.5 h for multiplex PCRs and for 1.5 h for uniplex PCRs in 2% agarose gels prepared in 0.5×Tris-borate-EDTA (TBE) buffer.

The presence of *E. coli* virulence genes were examined by PCR using primers and PCR conditions as documented by Chapman et al. (2006). Twelve sets of multiplex PCRs and six individual PCRs were used to detect the presence of 58 virulence genes from *E. coli* pathotypes known to cause intestinal and extra-intestinal disease in humans and animals (Table 2).

3. Results and discussion

3.1. Antibiotic resistance phenotypes of E. coli isolates

The results demonstrate the high individual and multiple resistance to antibiotics in *E. coli* and the 99 isolates showed resistance to all 15 antibiotics tested (Table 3). Overall, resistance was most frequently observed to tetracycline (77.8%), sulphafurazole (60.6%), ampicillin/amoxicillin (50.5%), trimethoprim (51.5%), chloramphenicol (43.4%), streptomycin (39.4%), nalidixic acid (34.3%) and gentamicin (24.2%). *E. coli* isolates also displayed resistance to fluoroquinolones (ciprofloxacin 16.2%, norfloxacin 17.2%, and enrofloxacin 21.2%), in which chicken isolates showed the highest rates of resistance to these

Table 1
Summary of 3 multiplex (sets 1–3) and 3 uniplex (sets 4–6) primer sets for the amplification of the 22 antimicrobial resistance genes

Primer set	Gene name	Antimicrobial resistance	Primers	DNA sequence 5' → 3'	Amplified product (bp)	Primer concentration
1	*sull*	Sulfonamide	sull-F	TTCGGCATTCTGAATCTCAC	822	0.56 μM
			sull-R	ATGATCTAACCCTCGGTCTC		0.56 μM
	SHV	Beta-lactam	blaSHV-F	TCGCCTGTGTATTATCTCCC	768	0.56 μM
			blaSHV-R	CGCAGATAAATCACCACAATG		0.56 μM
	cat1	Chloramphenicol	CATI-F	AGTTGCTCAATGTACCTATAACC	547	0.28 μM
			CATI-R	TTGTAATTCATTAAGCATTCTGCC		0.28 μM
	dhfrV	Trimethoprim	dhfrV-F	CTGCAAAAGCGAAAAACGG	432	0.28 μM
			dhfrV-R	AGCAATAGTTAATGTTTGAGCTAAAG		0.28 μM
	floR	Florfenicol	floR-F	TATCTCCCTGTCGTTCCAG	399	0.28 μM
			flor-R	AGAACTCGCCGATCAATG		0.28 μM
	aadA	Aminoglycoside	aadA-F	TGATTTGCTGGTTACGGTGAC	284	0.28 μM
			aadA-R	CGCTATGTTCTCTTGCTTTTG		0.28 μM
	OXA	Beta-lactam	blaOXA-F	GCAGCGCCAGTGCATCAAC	198	0.28 μM
			blaOXA-R	CCGCATCAAATGCCATAAGTG		0.28 μM
2	TEM	Beta-lactam	blaTEM-F	GAGTATTCAACATTTTCGT	857	0.56 μM
			blaTEM-R	ACCAATGCTTAATCAGTGA		0.56 μM
	cmlA	Chloramphenicol	cmlA-F	CCGCCACGGTGTTGTTGTTATC	698	0.28 μM
			cmlA-R	CACCTTGCCTGCCCATCATTAG		0.28 μM
	CITM	AmpC's	CITM-F	TGGCCAGAACTGACAGGCAAA	462	0.28 μM
			CITM-R	TTTCTCCTGAACGTGGCTGGC		0.28 μM
	ereA	Macrolide	ere(A)-F	GCCGGTGCTCATGAACTTGAG	419	0.28 μM
			ere(A)-R	CGACTCTATTCGATCAGAGGC		0.28 μM
	dhfrI	Trimethoprim	dhfrI-F	AAGAATGGAGTTATCGGGAATG	391	0.28 μM
			dhfrI-R	GGGTAAAAACTGGCCTAAAATTG		0.28 μM
	aac(3)-I	Aminoglycoside	aac(3)-I-F	ACCTACTCCCAACATCAGCC	157	0.56 μM
			aac(3)-I-R	ATATAGATCTCACTACGCGC		0.56 μM
3	*aphA-1*	Aminoglycoside	aphA-I-F	ATGGGCTCGCGATAATGTC	600	0.28 μM
			aphA-1-R	CTCACCGAGGCAGTTCCAT		0.28 μM
	MOXM	AmpC's	mox-1	GCTGCTCAAGGAGCACAGGAT	520	0.28 μM
			mox-2	CACATTGACATAGGTGTGGTGC		0.28 μM
	DHAM	AmpC's	DHA-1	AACTTTCACAGGTGTGCTGGGT	405	0.28 μM
			DHA-2	CCGTACGCATACTGGCTTTGC		0.28 μM
	EBCM	AmpC's	MR-1	TCGGTAAAGCCGATGTTGCGG	302	0.28 μM
			MR-2	CTTCCACTGCGGCTGCCAGTT		0.28 μM
	aac(3)-IV	Aminoglycosides	aac(3)-IV-F	CTTCAGGATGGCAAGTTGGT	286	0.28 μM
			aac(3)-IV-R	TCATCTCGTTCTCCGCTCAT		0.28 μM
	FOXM	FOXM	fox-1	AACATGGGGTATCAGGGAGATG	190	0.28 μM
			fox-2	CAAAGCGCGTAACCGGATTGG		0.28 μM
4	*tetA*	Tetracycline	tet(A)-F	GTGAAACCCAACATACCCC	887	0.5 μM
			tet(A)-R	GAAGGCAAGCAGGATGTAG		0.5 μM
5	*tetB*	Tetracycline	tet(B)-F	CCTTATCATGCCAGTCTTGC	773	0.5 μM
			tet(B)-R	ACTGCCGTTTTTTCGCC		0.5 μM
6	*tetC*	Tetracycline	tet(C)-F	ACTTGGAGCCACTATCGAC	880	0.5 μM
			tet(C)-R	CTACAATCCATGCCAACCC		0.5 μM

Forward and reverse primer sequence for each ARG, together with the size of the expected amplicon products are also shown (J. Chin, personal communication, 2006).

antibiotics (52.6–63.2%). In contrast to this study, resistance to ciprofloxacin in *E. coli* isolates from animal sources was reported as either low (Teshager et al., 2000) or non-existent (Meng et al., 1998; Klein and Bulte, 2003; Schroeder et al., 2003) in developed countries, perhaps due to restricted uses of fluoroquinolones in animal husbandry in these countries. In a country such as Canada, fluoroquinolones are not registered for use in pigs, therefore *E. coli* isolated from this source showed very little resistance to these antibiotics (Boerlin et al., 2005). Fluoroquinolones are critically important for treating serious infections in humans, and the likelihood that resistance to fluoroquinolones in *E. coli* was induced by the use of these antibiotics in food animals is a concern.

The *E. coli* isolates from chicken and pork showed a greater degree of resistance than those from beef, reflecting the higher use of antibiotics in intensive poultry and pig farming. In addition, *E. coli* isolates from chicken faeces had a similar antibiotic resistance distribution to chicken strains, except fluoroquinolone and amoxicillin/clavulanic acid, where resistance in chicken isolates was much higher. In this study, chicken faeces were collected from very young chickens (less than 1 month) whereas chicken meat from the market was originated from much older birds. The difference in ages of the chickens in our study could explain the different resistance levels as older chicken have longer exposure periods to in-feed antibiotics and therefore a greater possibility of their microbial flora developing resistance. Though resistance rates of *E. coli* from shellfish were less than that of meat and poultry, shellfish isolates displayed resistance to all 15 antibiotics tested. The resistance of *E. coli* isolates in shellfish to different antibiotic classes suggests that these *E. coli* strains were generated from different sources in the contaminated water environment where shellfish inhabit.

There were 83.8% of isolates that were resistant to one or more antibiotics, and multi-resistance was observed in all sources (61.6%) with rates up to 75% in pork, 89.5% in chicken, and 95% in chicken faeces (Table 3). Resistance to more than 10 antibiotics was also detected in chicken, pork and shellfish isolates, and there were 9.1% isolates which displayed resistance to 12–14 antibiotics. The finding of high levels of antibiotic resistance in *E. coli* isolates implies that *E. coli* from Vietnamese raw food may play an important role as reservoirs for the resistance genes and be a key source for transfer of resistance to other important human pathogens.

3.2. Antibiotic resistance genes in E. coli isolates

Genes responsible for a variety of antibiotic resistance characteristics have been investigated by multiplex and uniplex PCRs from 38 *E. coli* isolates. The results showed good correlation between antibiotic

Table 2
List of 58 virulence genes which were detectable by multiplex and uniplex PCRs in this study and their description/function (Chapman et al., 2006)

PCR set	Virulence gene(s)/ activity	Description/function
I	*fimH*[a]	D-Mannose-specific adhesin, type 1 fimbriae
	papEF	Minor tip pilins, connect PapG PapG to shaft (PapA)
	papA	Major structural subunit of pilus associated with pyelonephritis (P fimbriae), defines F antigen
	*kpsMT*III	Group III capsular polysaccharide synthesis (e.g., K3, K10, and K54)
	ibeA[a]	Invasion of brain endothelium
	PAI	Pathogenicity-associated island, provides mechanism for coordinate horizontal transfer of VF genes between lineages
II	*fyuA*[a]	*Yersinia*siderophore receptor (ferric yersiniabactin uptake)
	bmaE[a]	M-agglutinin subunit
	sfa/focDE	Central region of *sfa*(S fimbriae) and *foc*(F1C fimbriae) operons
	iutA[a]	Ferric aerobactin receptor (iron uptake/transport)
	papG allele III[a]	Cystitis-associated (*prs* or *pap-2*) *papG* variant (*papG*: Gal (1-4)Gal-specific pilus tip adhesin molecule)
	*kpsMT*K1	Specific for K1 (group II) *kpsMT*
III	*hlyA*	α-Hemolysin
	rfc	O4 lipopolysaccharide synthesis
	nfaE	Non-fimbrial adhesin I assembly and transport
	papG allele I	(Rare) J96-associated *papG* variant
	*kpsMT*II	Group II capsular polysaccharide synthesis (e.g., K1, K5, and K12)
	papC	Pilus assembly, central region of *pap* operon
IV	*cvaC*	Colicin V, conjugative plasmids (*traT*, *iss*, and antimicrobial resistance)
	cdtB	Cytolethal distending toxin
	focG	Pilus tip molecule, F1C fimbriae fimbriae (sialic acid specific)
	traT[a]	Surface exclusion, serum survival
	papG allele II	Pyelonephritis-associated *papG* variant
V	*papG* allele I	(Rare) J96-associated *papG* variant
	papG alleles II and III	
	afa/draBC	Central region of Dr antigen-specifific fimbrial fimbrial and afimbrial adhesin operons (e.g., AFA, Dr, and F1845)
	cnf1	Cytotoxic necrotizing factor 1
	sfaS	Pilus tip adhesin, S fimbriae (sialic acid specific)
	kpsMT"K5"	Specific for non-K1 and non-K2 group II *kpsMT*
VI	univcnf	Universal primer for cytotoxic necrotizing factor 1
	iha[a]	Novel nonhemagglutinin adhesin (from O157:H7 and CFT073)
	$iroN_{E.coli}$[a]	Novel catecholate siderophore
	ompT	Outer membrane protein A and T (protease)
	papG allele I'	*papG* variant identified in canine urine
	iss[a]	Serum survival gene
	ireA	Iron-regulated element, a siderophore receptor
VII	*ehxA*	Enterohemolysin
	eaeA	Intimin
	stx1	Shiga toxin I
	stx2	Shiga toxin II
VIII	*eltA*	Heat-labile toxin
	fasA	F6 fimbrial adhesion
	STb	Heat-stable enterotoxin b
IX	*faeG*	F4 fimbrial adhesion
	fanC	F5 fimbrial adhesion
	STa	Heat-stable enterotoxin a
X	*fedA*	F18 fimbrial adhesion
	F41	Fimbrial adhesion
XI	*aah*[a]	Autotransporter adhesin heptosyltransferase encoding AAH protein which modifies AIDA-I adhesin
	aidA AIDA-I[a]	Adhesin involved in diffuse adherence, consisting of AIDA-I (*orfB*) and AIDA[c] (*orfB*c)
	aidA AIDA[c]	
XII	*chuA*[a]	Gene required for heme transport in EHEC O157:H7
	yjaA[a]	Identified in *E. coli* K12, function currently unknown
	TSPE4.C2[a]	Anonymous DNA fragment
XIII	*east1*[a]	EaggEC heat-stable enterotoxin
XIV	*cdt*	Cytolethal distending toxin
XV	*paa*	Porcine A/E-associated gene
XVI	*saa*	STEC autoagglutinating adhesion
XVII	*ipaH*	Invasion plasmid antigen
XVIII	*bfpA*	Type IV bundle-forming pili

[a] Genes detected in 38 *E. coli* food isolates from Vietnam.

resistance phenotype and genotypes in these *E. coli* isolates (Table 4). More than one gene encoding the same resistance was detected in one strain: two isolates from chicken contained tetracycline resistance genes *tetA*+*tetC* and *tetB*+*tetC*, one isolate from chicken and another isolate from chicken faeces contained chloramphenicol resistance genes *cat1* and *cmlA*. There were 84.2% of the tested isolates which contained the beta-lactamase bla_{TEM} gene. Our findings are similar to studies in other countries showing that *E. coli* strains from food of animal origin had a unique beta-lactamase bla_{TEM} gene (Brinas et al., 2002; Guerra et al., 2003). In contrast, other beta-lactamase genes such as SHV-type and OXA-type were not detected in any isolates in this study.

Plasmid-mediated *ampC* beta-lactamase genes were observed in some *E. coli* isolates as a positive PCR result was detected with MOXM family-specific primers in chicken and pork isolates, suggesting the presence of MOX-1, MOX-2, CMY-1, or CMY-8 to CMY-11 genes in these isolates (Perez-Perez and Hanson, 2002). In addition, an expected band has been observed in a beef isolate with DHAM family-specific primers, indicating the isolate contained DHA-1 or DHA-2 genes (Perez-Perez and Hanson, 2002). Therapeutic options may be limited for infections caused by Gram-negative bacteria overexpressing plasmid-mediated AmpC beta-lactamases because such overexpression can lead to resistance to most beta-lactam antibiotics except for cefepime, cefpirome and carbapenems (Perez-Perez and Hanson, 2002). In addition, plasmids encoding AmpC enzymes often carry multiple resistances and there is evidence of spread of plasmid-mediated AmpC beta-lactamases genes between organisms (Bauernfeind et al., 1997; Winokur et al., 2001; Philippon et al., 2002; Yan et al., 2004). Further studies of the distribution of *ampC* genes are therefore necessary.

The rapid spread of tetracycline resistant determinants within a bacterial population is due to the location of tetracycline genes on mobile elements (Chopra and Roberts, 2001; Roberts, 2003; Sunde and Nordstrom, 2006). In this study, it was found that tetracycline resistance genes did spread in *E. coli* populations. The *tetA* gene was the most prevalent of the tetracycline resistance genes detected (71.1% of the isolates), followed by *tetB* (18.4%). These two genes were reported to be predominant in *E. coli* isolates from livestock and food animals in other countries (Guerra et al., 2003; Lanz et al., 2003; Sengelov et al., 2003; Bryan et al., 2004; Saenz et al., 2004; Boerlin et al., 2005). Genes which are responsible for resistance to streptomycin (*aadA*), chloramphenicol (*cmlA*), sulfonamides (*sulI*), trimethoprim (*dhfrV*) and kanamycin (*aphA-1*) were also widely distributed with the

Table 3
Percentage of *E. coli* isolates from different sources which were resistant to different antibiotics

Antibiotics	Percentage of resistance					
	Pork (20)	Beef (20)	Chicken (19)	Chicken faeces (20)	Shellfish (20)	Total (99)
AMP	55.0	20.0	84.2	65.0	30.0	50.5
AMX	55.0	20.0	84.2	65.0	30.0	50.5
AMC	0.0	0.0	15.8	0.0	5.0	4.0
TET	100.0	60.0	84.2	95.0	50.0	77.8
SUL	70.0	10.0	94.7	95.0	35.0	60.6
KAN	10.0	0.0	15.8	25.0	5.0	11.1
GEN	25.0	0.0	47.4	45.0	5.0	24.2
STR	65.0	15.0	63.2	30.0	25.0	39.4
NOR	15.0	0.0	57.9	5.0	10.0	17.2
ENR	20.0	0.0	63.2	15.0	10.0	21.2
CIP	15.0	0.0	52.6	5.0	10.0	16.2
NAL	30.0	0.0	68.4	50.0	25.0	34.3
CHL	50.0	20.0	57.9	65.0	25.0	43.4
CEF	5.0	5.0	31.6	10.0	20.0	14.1
TMP	60.0	20.0	63.2	90.0	25.0	51.5
Resistance to≥1 antibiotic	100	65.0	100.0	100.0	55.0	83.8
Multi-resistance[a]	75.0	15.0	89.5	95.0	35.0	61.6

[a] Resistance to at least 3 different classes of antibiotics.

Table 4
Summary of antibiotic resistance profiles compared with the presence of antibiotic resistance and virulence genes in *E. coli* isolates

Isolate name	Food source	Antibiotic resistance characteristics		Virulence genes
		Resistance/intermediate resistance patterns	Resistance genes	
E/C/3a	Chicken	AMP, CIP, TET, GEN, CHL, SUL, TMP, NOR, STR, KAN, NAL, ENR, AMX/CEF, AMC	*tetA*, *aadA*, *dhfrV*, TEM, *cmlA*, *aphA-1*, *aac(3)-IV*	*fimH*, *bmaE*, *traT*, *east1*
E/C/4a	Chicken	AMP, CIP, TET, GEN, CHL, SUL, NOR, NAL, ENR, AMX/ CEF, AMC, TMP, STR, KAN	*tetA*, *tetC*, *aadA*, TEM, *cmlA*, *aac (3)-IV*	*fimH*, *bmaE*
E/C/5a	Chicken	AMP, CIP, TET, SUL, TMP, NOR, STR, NAL, ENR, AMX, AMC, CEF	*tetA*, *aadA*, *dhfrV*, TEM	*fimH*, *bmaE*, *iutA*, *traT*, *Iha*, *iroN*$_{E.coli}$, *iss*, *aah* (*orfA*), TSPE4.C2
E/C/9b	Chicken	AMP, CIP, TET, GEN, CHL, SUL, TMP, NOR, STR, KAN, NAL, ENR, AMX, CEF/AMC	*tetA*, *aadA*, *dhfrV*, TEM, *cmlA*, *dhfrI*, *aphA-1*	*fimH*, *ibeA*, *bmaE*, *traT*, *chuA*, *east1*
E/C/11a	Chicken	AMP, TET, CHL, SUL, TMP, STR, NAL, AMX/CEF, AMC, ENR	*tetA*, *dhfrV*, TEM	*fimH*, *bmaE*, AIDA-I (*orfB*)
E/C/13a	Chicken	AMP, CIP, TET, GEN, SUL, NOR, STR, NAL, ENR, AMX/CEF, AMC, TMP	*tetA*, TEM	*fimH*, *bmaE*, *iutA*, *traT*, *chuA*, TSPE4.C2, *east1*
E/C/15a	Chicken	AMP, TET, SUL, TMP, STR, NAL, ENR, AMX/CEF, AMC	*tetB*, *tetC*, *sulI*, TEM, MOXM	*fimH*, *bmaE*, *east1*
E/C/16a	Chicken	AMP, CIP, TET, GEN, CHL, SUL, TMP, NOR, NAL, ENR, AMX, CEF/AMC, KAN	*tetB*, *cat1*, *sulI*, TEM, *dhfrI*, MOXM	*fimH*, *bmaE*, TSPE4.C2, *east1*
E/C/17a	Chicken	AMP, TET, GEN, CHL, SUL, TMP, NOR, KAN, NAL, ENR, AMX, STR/CEF, CIP, AMC	*tetA*, *aadA*, *cat1*, *sulI*, TEM, *cmlA*, *aphA-1*, *aac(3)-IV*	*fimH*, *bmaE*, TSPE4.C2, *east1*
E/C/20a	Chicken	AMP, CIP, TET, GEN, CHL, SUL, NOR, NAL, ENR, AMX/ CEF, AMC, TMP, STR	*tetB*, *aadA*, TEM, *cmlA*, *aac(3)-IV*	*fimH*, *bmaE*, *east1*
E/C/21a	Chicken	AMP, CIP, TET, GEN, CHL, SUL, TMP, NOR, STR, NAL, ENR, AMX, CEF/AMC, KAN	*tetB*, *aadA*, *sulI*, *cat1*, TEM, *dhfrI*, MOXM	*fimH*, *bmaE*, TSPE4.C2
E/C/24a	Chicken	AMP, TET, GEN, SUL, TMP, AMX, CHL, STR/CEF, AMC, NAL, ENR	*tetA*, *aadA*, TEM, *cmlA*, *aac(3)-IV*	*fimH*, *bmaE*, *fyuA*, AIDA (*orfB*)
E/C/25b	Chicken	AMP, CIP, TET, CHL, SUL, TMP, NOR, STR, NAL, ENR, AMX, AMC	*tetA*, *aadA*, TEM, *cmlA*	*fimH*, *bmaE*, *traT*, *iroN*$_{E. coli}$, *iss*, AIDA (*orfB*)
E/C/29a	Chicken	AMP, TET, SUL, TMP, AMX/ CEF, AMC, STR	*tetA*, *aadA*, *sulI*, TEM, *dhfrI*	*fimH*, *bmaE*, AIDA (*orfB*), TSPE4.C2
E/P/15a	Pork	AMP, TET, GEN, CHL, SUL, TMP, STR, KAN, AMX/NAL, ENR	*tetB*, *aadA*, TEM, *cmlA*, *aphA-1*, *aac(3)-IV*	*traT*, AIDA(*orfB*)
E/P/18a	Pork	AMP, TET, GEN, SUL, NAL, ENR, AMX, STR/CEF, NOR	*tetA*, *aadA*, TEM, MOXM	*fimH*, *bmaE*, *fyuA*, *papG* III, AIDA (*orfB*), TSPE4.C2, *east1*
E/P/20a	Pork	AMP, TET, CHL, SUL, TMP, STR, AMX/CEF, AMC	*tetA*, *aadA*, TEM, *cmlA*	*fimH*, *bmaE*, *east1*
E/P/27a	Pork	CIP, TET, GEN, SUL, TMP, NOR, NAL, ENR, STR/CEF, AMC, AMP, AMX	*tetA*, *aadA*	*fimH*, TSPE4.C2
E/P/43a	Pork	AMP, TET, GEN, SUL, TMP, AMX, STR/CHL	*tetA*, *aadA*, TEM	*fimH*, *bmaE*, AIDA (*orfB*), *yjaA*
E/P/48a	Pork	AMP, TET, CHL, SUL, TMP, STR, NAL, AMX/CEF, AMC, ENR	*tetA*, *aadA*, *dhfrV*, TEM, *cmlA*	*fimH*, *bmaE*, *fyuA*, TSPE4.C2
E/P/49a	Pork	AMP, CIP, TET, GEN, CHL, SUL, TMP, NOR, STR, KAN, NAL, ENR, AMX/CEF, AMC	*tetA*, *aadA*, *dhfrV*, TEM, *cmlA*, *apha-1*	*fimH*, *bmaE*, AIDA (*orfB*), TSPE4.C2
E/P/25a	Pork	AMP, CIP, TET, CHL, SUL, TMP, NOR, STR, NAL, ENR, AMX/CEF, AMC	*tetB*, *aadA*, *cat1*, *sulI*, TEM, *dhfrI*	*fimH*, *bmaE*, *iutA*, *traT*
E/B/16a	Beef	AMP, TET, CHL, SUL, TMP, STR/AMC	*tetA*, *dhfrV*, TEM	*fimH*
E/B/17b	Beef	AMP, TET, CHL, TMP, STR, AMX/CEF, AMC,NAL, ENR	*tetA*, *dhfrV*, TEM	AIDA-I(*orfB*)
E/B/46	Beef	AMP, TET, CHL, SUL, TMP, STR, AMX/CEF, AMC	*tetA*, *dhfrV*, TEM	*fimH*, *bmaE*, TSPE4.C2
E/F/3	Chicken faeces	AMP, TET, GEN, CHL, SUL, TMP, STR, NAL, ENR, AMX/ CEF, NOR	*tetA*, *aadA*, *sulI*, TEM, *dhfrI*	*fimH*, *bmaE*
E/F/8	Chicken faeces	AMP, TET, GEN, CHL, SUL, TMP, KAN, NAL, ENR, AMX/ CEF, AMC, STR	*tetA*, *aadA*, *sulI*, TEM, *cmlA*	AIDA-I(*orfB*)
E/F/9	Chicken faeces	AMP, TET, GEN, CHL, SUL, TMP, NAL, AMX, CEF/AMC, STR, ENR	*tetA*, *aadA*, *cat1*, *sulI*, TEM, *cmlA*	*fimH*, *bmaE*
E/F/13	Chicken faeces	TET, GEN, CHL, SUL, TMP, KAN, NAL/CEF, STR, ENR	*tetA*, *aadA*, *sulI*, *cmlA*, *aphA-1*	*fimH*, *bmaE*, *traT*
E/F/16	Chicken faeces	TET, GEN, CHL, SUL, TMP, KAN, NAL/CEF, STR, ENR	*tetA*, *aadA*, *sulI*, *cmlA*, *aphA-1*	*fimH*, *bmaE*, *traT*
E/F/20	Chicken faeces	TET, GEN, CHL, SUL, TMP, KAN, NAL/STR, ENR	*tetA*, *aadA*, *sulI*, *cmlA*, *aphA-1*	*fimH*, *bmaE*
E/F/25	Chicken faeces	TET, CHL,SUL, TMP, NOR, NAL, ENR, CIP/CEF, STR	*tetA*, *aadA*, *sulI*, *cmlA*	*fimH*, *bmaE*
E/SF/1a	Shellfish	AMP, TET, CHL, SUL, TMP, STR,NAL, AMX/CEF, AMC, ENR	*tetA*, *cat1*, *sulI*, TEM	*fimH*, *bmaE*, TSPE4.C2
E/SF/6a	Shellfish	AMP, CIP, TET, CHL, SUL, TMP, NOR, STR, NAL, ENR, AMX/AMC	*tetB*, *aadA*, TEM, *cmlA*	*fimH*, *bmaE*, *yjaA*, *east1*
E/SF/10a	Shellfish	AMP, CIP, TET, SUL, TMP, NOR, STR, KAN, NAL, ENR, AMX, CEF/AMC	*tetB*, *dhfrV*, TEM, *aphA-1*	*fimH*, *bmaE*, AIDA (*orfB*), TSPE4.C2
E/SF/29	Shellfish	AMP, TET, GEN, SUL, CEF, TMP, STR, NAL, AMX, CEF, AMC/CHL, ENR	*cat1*, *sulI*, TEM	*fimH*, *fyuA*, *chuA*, TSPE4.C2
E/SF/47a	Shellfish	AMP, TET, CHL, SUL, TMP, STR, AMX, CEF/AMC	*tetA*, TEM	*fimH*, *bmaE*, TSPE4.C2
E/B/41	Beef	/CEF, AMP, AMX	DHAM	*fimH*, *bmaE*, AIDA (*orfB*), TSPE4.C2

rates of 68.4%, 42.1%, 39.5%, 26.3% and 23.7% respectively. Other resistance genes including *cat1* (chloramphenicol resistance), *dhfrI* (trimethoprim resistance) and *aac(3)-IV* (gentamicin resistance) were also detected (18.4–10.5%) in this *E. coli* collection. High level of *E. coli* resistance in food sources should be a cause for concern as this organism has high propensity to disseminate antimicrobial resistance genes (WHO, 1997).

3.3. Virulence genes in E. coli isolates

PCR amplification has been a sensitive and valuable method for detection of virulence genes in *E. coli* strains (Nataro and Kaper, 1998; Osek et al., 1999; Paton and Paton, 2002; Chen et al., 2004). In this study, 38 *E. coli* isolates were examined for the presence of virulence genes using the method of Chapman et al. (2006). All tested isolates were positive for at least one virulence gene with 16 out of 58 specific genes detected (Table 4). The wide range of ExPEC-associated virulence markers of different virulent functions, including *bmaE*, *fimH*, *fyuA*, *iroN*$_{E. coli}$, *iutA*, *ibeA*, *iss*, *traT*, and *papG* III has been detected, in which *fimH* and *bmaE* genes were dominant and were detected in 92.1% and 84.2% of isolates from all sources respectively. The high prevalence of the *fimH* gene obtained in this study was consistent with previous reports (Johnson and Stell, 2000; Bekal et al., 2003) that this gene was present in all *E. coli* isolates of different pathotypes and also in non-pathogenic *E. coli*. In adapting to a pathogenic environment, the genetic variation in the *fimH* gene can change the tropism of *E. coli*, shifting it toward a virulent phenotype (Sokurenko et al., 1998). The virulence genes TSPE4.C2, *aidA* AIDA-I (*orfB*), *east1*, *traT*, were also present at a moderate to high rate (42.1%,

31.6%, 26.3%, and 23.7% respectively). Notably, the *east1* gene which is involved in human outbreaks of diarrhoea (Vila et al., 1998; Zhou et al., 2002) was found with high frequency in all food sources (20–50%). The other virulence genes detected in this study include *fyuA*, *iutA*, *chuA*, *yjaA*, *iss*, *iroN*$_{E.\ coli}$, *ibeA*, *aah* (*orfA*), *iha*, and *papG* allele III (10.5–2.6%). As strains of the same pathotype normally carry the same virulence determinants involved in infection (Bekal et al., 2003), the detection of 58 virulence genes associated with representative pathotypes of *E. coli* in this study allow detection of the presence of certain pathotypes in these 38 *E. coli* isolates. In this case, the *stx1* and *stx2* virulence genes associated with the STEC pathotype were not detected, and heat-stable toxin (ST) and heat-labile toxin (LT) genes specific to ETEC were not observed. These results imply that none of these 38 isolates belongs to STEC or ETEC pathotypes. In contrast, the STEC pathotype has been found in raw food, especially beef in other countries and mostly in industrialised countries (Parma et al., 2000; Kumar et al., 2001; Guth et al., 2003; Blanco et al., 2004; Conedera et al., 2004; Barlow et al., 2006; Carney et al., 2006; Samadpour et al., 2006). There were multi-resistant isolates which contained from 6 to 9 virulence genes. *E. coli* strains in this study may not possess pathogenic functionality due to lack of appropriate virulence gene combinations (Chapman et al., 2006). However, bacterial virulence can be increased and new pathotypes with new combinations of virulence factors may occur as virulence genes can be transferred between organism populations over time, especially since the genome of *E. coli* is of highly plasticity (Kuhnert et al., 2000), and the problem would be considerable if this virulence acquisition occurred in multi-resistant strains.

The average number of antibiotic resistance genes and virulence genes from different origins were calculated. The average number of antibiotic resistance genes in different sources were: chicken (5.3), chicken faeces (5.0), pork (4.5), shellfish (3.4), and beef (2.5), whereas the average number of virulence genes were: chicken (4.4), pork (3.8), shellfish (3.6), beef (2.3) and chicken faeces (2.1). Comparing to beef isolates, chicken and pork isolates harboured more antibiotic resistance genes and virulence genes. Chicken faeces also contained high number of antibiotic resistance genes. Interestingly, it was observed that compared to other sources, *E. coli* in chickens showed a higher frequency of resistance phenotype, they also exceed other sources in terms of degree of antibiotic resistance genes and virulence genes. In addition, among 38 isolates which were investigated for the presence of antibiotic resistance genes and virulence genes, isolates which contained the maximum number of antibiotic resistance genes (isolate E/C/17a) and virulence genes (isolate E/C/5a) also belonged to chicken isolates (Table 4). Using all the results together, it could be concluded that chicken meat is the most risk-associated food source in terms of antibiotic resistance and virulence potential. The spread of antimicrobial agents given to poultry might be the reason for high rates of resistance and overcrowded population in poultry husbandry may contribute to the spread of antibiotic and virulence genes between populations.

This study has focused primarily on the characterization of antibiotic resistance genes found in *E. coli* isolated from raw foods in Vietnam. A parallel study on virulence genes in these isolates has confirmed an absence of combinations of virulence factors that signal the covert presence of zoonotic strains of *E. coli* such as O157 and other STECs amongst the isolates. However, the strains were not analysed for carriage of signature virulence genes found in avian pathogenic *E. coli* (APECs). The importance of this study is the finding that enteric bacteria in Vietnamese food samples are significant reservoirs of antibiotic resistance genes. In the light of recent epidemiological findings that urinary tract infections in humans may be associated with poultry consumption (Manges et al., 2007), it endorses the need for more rigorous surveillance and improved farming practises that can reduce the carriage of ARGs and thereby minimize the likelihood of horizontal gene transfers of these antimicrobial resistance genes to other microbes in the food chain. Training for food handlers on safe food handling and proper cooking are therefore important to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from raw foods. Additionally, it is recommended that antibiotic usage in animal feed must be strongly regulated. There is still a big gap in understanding the genetic background of antibiotic resistance and virulence of enteric bacteria from food. Studies with comprehensive collections of samples are urgently needed to establish better measures for preventing foodborne disease.

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