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Original Article

Molecular classification and drug resistance analysis of *Escherichia coli* isolated from poultry in China

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Abstract: We aimed to understand the distribution of *Escherichia coli* in poultry and to reveal the virulence factors, the drug resistance and molecular epidemic regularity and characteristics of isolate strains from 6 provinces in China and to complete the characteristics of *E. coli* for the risk assessment. A total of 87 *E. coli* isolates were analyzed with 7 virulence genes by PCR drug sensitivity test in 13 kinds of antimicrobial agents and analyzed with PFGE and MLST genotyping. The PFGE genotyping of 87 isolates yielded 75 PFGE type. MLST analysis of isolates identified the 39 STs, the 7 housekeeping genes had the different variation. The most prevalent virulence genes were iucD (74.7%), followed by iss (55.2%), Irp2 (43.7%), tsh (28.7%), cva (19.5%), papC (9.2%) and vat (8.1%). All isolates were resistant to two or three antimicrobial agents highly resistant to SXT, TE (85.06%), SF (83.91%), AM (66. 67%), to fluoroquinolones (ENR, 63.22%, NOR, 50.57%) and to GM (57.47%). *E. coli* strains resistant spectrum was wide gene was polymorphism the distribution had a certain timeliness and regional in part region of China. These were a solid foundation for the epidemiological investigation and traceability laid.

Keywords: *Escherichia coli*, virulence genes, resistance gene, drug resistance, pulsed-field gel electrophoresis, multilocus sequence analysis, poultry

Introduction

*Escherichia coli is one of the normal microflo*ras in humans and animals intestine. Some of them is a well-known pathogen that cause diarrhea. hemorrhagic colitis and hemolytic uremic syndrome [1]. Most cases have been attributed to O157:H7, but the importance of non-O157 STEC is also increasingly recognized [2].

E. coli possesses a number of virulence factors. Some of which related to *E. coli* pathogenicity. The *Yersinia* high-pathogenicity island (HPI) carrying *Irp2* (encoding the siderophore yersiniabactin) is also present in certain non-O157 STEC lineages which was previously reported only in *stx2e* carrying human isolates [3]. *papC* gene encode the adherence factor intimin and i*cuD* is lysine monoxygenase encoding the aerocin [4]. Temperature sensitive hemagglutinin (*tsh*) and cavitation automatic transport toxins (*vat*) are automatically transfer protein family. *Iss* genes (encoding the outer membrane protein) is an important pathogenic factor of *E. coli*, it can enhance the resistance of *Escherichia coli* in serum or be associated with bacterial resistance to complement effect [5-7]. Some *Iss* genes containing ColV plasmid can produce colicin V (*cva*), which have close relationship with chicken *Escherichia coli* disease.

Pulsed-field gel electrophoresis (PFGE) is a DNA fingerprinting method that can distinguish different strains within a species by comparing genotypic characteristics. It is an analytical technique in the field of molecular epidemiology [8, 9], where this method has been traditionally used for identifying the route and source of infection for specifying bacteria that are responsible for food illness [10]. PFGE has extremely high sensitivity reproducibility and discrimination ability compared with other MLST methods [11].

Table 1. PCR primers used for *E. coli* virulence genes amplification

Targets	Primer	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Reference
iucD	*iucD*-F	ACAAAAAGTTCTATCGCTTCC	714	[17]
	iucD-R	CCTGATCCAGATGATGCTC		
irp2	*irp2*-F	AAGGATTCGCTGTTACCGGAC	280	[18]
	irp2-R	TCGTCGGGCAGCGTTTCTTCT		
papC	*papC*-F	TGATATCACGCAGTCAGTAGC	501	[19]
	papC-R	CCGGCCATATTCACATAA		
iss	*iss*-F	ATCACATAGGATTCTGCCG	309	[17]
	iss-R	CAGCGGAGTATAGATGCCA		
tsh	*tsh*-F	ACTATTCTCTGCAGGAAGTC	824	[17]
	tsh-R	CTTCCGATGTTCTGAACGT		
vat	*vat*-F	TCCTGGGACATAATGGTCAG	1000	[17]
	vat-R	GTGTCAGAACGGAATTGT		
cva	*cva*-F	TGGTAGAATGTGCCAGAGCAAG	1200	[17]
	cva-R	GAGCTGTTTGTAGCGAAGCC		

Multilocus sequence typing (MLST) has become the method of choice for typing epidemiologically important strains [12]. This method is based on determining short nucleotide sequences (450-500 bp) of several (five to seven) housekeeping genes that had undergone some evolutionary diversification leading to polymorphism [13]. MLST is highly discriminatory detecting even few nucleotide substitutions and enables easy automation of polymerase chain reaction (PCR) and sequence determination. Furthermore, it offers better standardization and lab-to-lab portability than its predecessor multilocus enzyme electrophoresis since it relies on sequence data, which are easily accessible in computer databases [14].

This study was designed to understand the distribution of *Escherichia coli* in large scale poultry population from 6 provinces (Shandong, Shanxi, Neimeng, Anhui, Henan and Chongqing) in China from 2000 to 2012 as well as reveal their virulence factors drug resistance molecular epidemic regularity and characteristics.

Materials and methods

E. coli isolates

87 isolates used in this study were randomly selected from the collection of Microbiology laboratory of Quality & Safety Risk Assessment for Animal Products of Ministry of Agriculture (CAHEC) in Qingdao, China. All these strains were isolated from 32 intensive poultry farms ranging from 10, 000 to 100, 000 poultry per farm in the above 6 provinces from 2000 to 2012: 2000 (13), 2008 (31), 2009 (5), 2010 (19), 2011 (16) and 2012 (3). All isolates were confirmed to be *E. coli* using API 20E biochemical test strips (bioMérieux, France). Sorbitol fermentation characteristic was examined using sorbitol-MacConkey agar (SMAC) (Oxoid, UK). The determination of O antigens was firstly carried out by testing for specific *E. coli* O groups of interest targeting group specific genes within the O-antigen gene cluster described by Deb Roy [15]. The entire coding sequence of the *fliC* gene was amplified by PCR with the primers *fliC*-F (5'-ATGGCACAAGTCATTAATACCCAAC-3') and *fliC*-R (5'-CTAACCCTGCAGCAGAGACA-3') reported by Qing M [16]. Serotypes of each isolate were determined by agglutination tests with anti-*Escherichia coli* sera (SSI Denmark).

Identification of virulence genes

For *E. coli* isolates 7 virulence-associated genes (*iucD, iss, vat, Irp2, papC, tsh* and *cva*) were sought as previously described by PCR Primers specific for these virulence-associated genes were shown in **Table 1**. PCR products were sequenced on ABI Prism 3100 automated sequencer (Applied Biosystems, USA) and were analyzed using NCBI BLAST program (http://www ncbi nlm nih gov/).

Antimicrobial susceptibility testing

Susceptibility of the isolates to antimicrobial agents was evaluated according to Clinical Laboratory Standards Institute (CLSI) guidelines [20] using the disc diffusion methodon Mueller-Hinton agar (Becton Dickinson, USA). Following 13 antimicrobial agents were tested, including chloramphenicol (florfenicol), penicillins (ampicillin and auge door-keeper), sulfonamides (sulfisoxazole and trimethoprim-sulfamethoxazole), cephems (cefotaxime), aminogly-

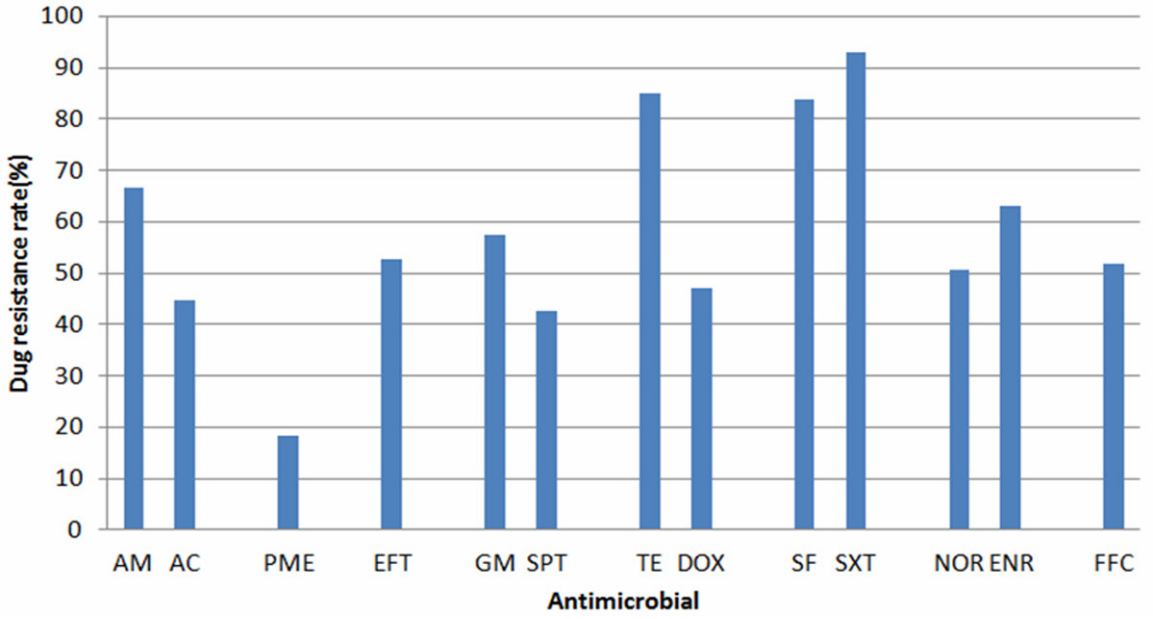


Figure 1. The dug resistance rate of all isolates for 13 kind of antimicrobial agents. Statistical test was only performed in all isolated. Antibiotics abbreviations are: AM, Ampicillin, AC, Auge door-keeper, PME, Polymyxin, EFT, Cefotaxime, GM, Gentamicin, SPT, Spectinomycin, TE, Tetracycline, DOX, Doxycycline, SF, Sulfisoxazole, SXT, Trimethoprim-sulfamethoxazole, NOR, Ofloxacin, ENR, Enrofloxacin, FFC, Florfenicol. PFGE typing.

cosides (gentamicin and spectinomycin), tetracyclines (tetracycline and doxycycline), fluoroquinolones (enrofloxacin and ofloxacin), and other (polymyxin). Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI, 2012) breakpoints when available *E. coli* ATCCR 25922 was used as quality control.

Pulsed-field gel electrophoresis

PFGE of the strains were performed using the non-O157 STEC subtyping protocol (www pulsenetinternational org) with some modifications. The bacteria genomic DNA was digested with 50 U of *Xba* I (Takara, China) at 37°C for 3 h. *Xbal*-digested *Salmonella enterica* serovar Braenderup H9812 was used as the DNA size marker, PFGE was repeated twice to determine reproducibility. For untypeable isolates, 50 μM thiourea (Sigma USA) was added to the 0 5× TBE buffer prior to PFGE run as described by Römling and Tümmler [21]. A contour-clamped homogenous electric field apparatus CHEF-Mapper (Bio-Rad, USA) was used. The pulse time was ramped from 2.16 s to 54.17 s over 19 h at 6.0 V/cm Gel images were captured with a Gel Documentation 2000 software (Bio-Rad, USA) and converted to Tiff files, then analyzed using BioNumerics software (Applied Maths Belgium).

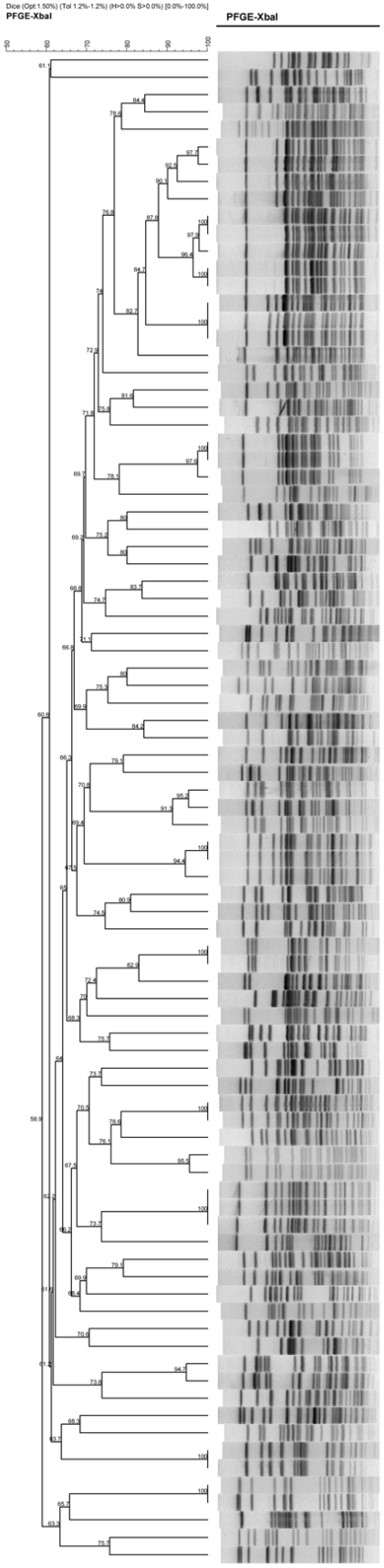
Multi-locus sequence typing

Multi-locus sequence typing (MLST) was performed according to the recommendations of the *E. coli* MLST website (http://mlst ucc ie/mlst/dbs/Ecoli) using 7 housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA* and *recA*). Alleles and sequence types (STs) were determined following the web site instructions [22]. A minimum spanning tree based on these STs was generated with BioNumerics software.

Results

Virulence gene

The results of the distribution of virulence determinants in *E. coli* isolates in relation were reported in **Figure 2**. All the 7 virulence factor genes sought were identified in at least 7 isolates. The most prevalent virulence genes were *iucD* (74.7%), followed by *iss* (55.2%), *Irp2* (43.7%), *tsh* (28.7%), *cva* (19.5%), *papC* (9.2%) and *vat* (8.1%). The ST23 isolates didn't exhibit the same virulence profiles. Only five different virulence genes were uniformly present in 7



Key	province	year	virulence gene	Antibiotic resistance	ST	PFGE-Pattern
AH08-8	anhui	2008	"iucD,iss"	"ENR,TE,GM,FFC,SF,SXT,AM"	10	0001
NM10-11	neimeng	2010	"iucD,vat"	"NOR,ENR,TE,GM,EFT,SF,SXT,AC,AM"	93	0002
NM10-12	neimeng	2010	"iucD,lrp2"	"NOR,ENR,TE,SPT,DOX,EFT,SF,SXT,AC,AM"	2309	0003
SD12-2	shandong	2012	"iucD,tsh,iss,vat"	"NOR,ENR,TE,GM,SPT,DOX,EFT,FFC,SF,SXT,AC,AM"	2505	0004
SD08-9	shandong	2008	iucD	"AM,EFT,GM,SPT,TE,FFC,SF,SXT,ENR"	354	0005
SD00-10	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM,GM"	23	0006
SD00-2	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM,GM,SPT"	23	0007
SD00-9	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM,SPT"	23	0008
SD00-4	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM,GM"	23	0009
SD00-5	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM,GM"	23	0010
SD00-8	shandong	2000	/	"NOR,TE,DOX,SF,SXT,AM,GM"	23	0010
SD00-6	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM"	23	0011
SD00-13	shandong	2000	"lrp2,iss"	"NOR,TE,DOX,SF,SXT,AM,GM"	23	0012
SD00-7	shandong	2000	"iucD,lrp2,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM,GM"	23	0012
SD00-1	shandong	2000	"iss,tsh"	"NOR,TE,DOX,SF,SXT,AM"	2505	0013
SD00-11	shandong	2000	"iss,tsh"	"NOR,TE,DOX,SF,SXT,AM"	2505	0013
SD00-12	shandong	2000	"iucD,iss,cva,tsh"	"NOR,TE,DOX,SF,SXT,AM"	2505	0013
SD08-16	shandong	2008	"iucD,lrp2,iss,cva"	"ENR,TE,GM,SPT,EFT,FFC,SF,SXT,AC,AM"	23	0014
SX10-3	shanxi	2010	"iucD,iss"	"NOR,ENR,TE,GM,SPT,FFC,SF,SXT,AC,AM"	93	0015
NM10-3	neimeng	2010	lrp2	"TE,DOX,SF,SXT,AC"	1125	0016
SD08-15	shandong	2008	"iucD,iss,cva"	"TE,GM,EFT,FFC,SF,SXT,AC,AM"	602	0017
SD11-7	shandong	2011	lrp2	"NOR,ENR,TE,PME,GM,SPT,EFT,SXT,AC"	156	0018
NM10-8	neimeng	2010	papC	"NOR,ENR,TE,GM,SPT,DOX,EFT,FFC,SF,SXT,AC,AM"	711	0019
NM10-9	neimeng	2010	"iucD,lrp2"	"TE,EFT,FFC,SF,SXT,AC,AM"	711	0019
NM10-4	neimeng	2010	"iucD,lrp2"	"ENR,TE,SF"	711	0020
AH08-2	anhui	2008	"iucD,lrp2"	"ENR,DOX,SF,SXT,AM"	1724	0021
AH08-7	anhui	2008	"iucD,tsh,iss"	"ENR,SF,SXT,AM"	115	0022
CQ11-2	chongqing	2011	"iucD,lrp2,iss"	"NOR,ENR,TE,GM,SPT,DOX,FFC,SF,SXT,AC"	10	0023
AH08-5	anhui	2008	lrp2	"TE,GM,DOX,EFT,FFC,SF,SXT,AM"	2165	0024
HN08-5	henan	2008	"iucD,lrp2"	"ENR,FFC,SXT"	539	0025
NM10-6	neimeng	2010	"iucD,lrp2,iss"	"NOR,ENR,TE,GM,EFT,FFC,SF,SXT,AC,AM"	602	0026
SD11-5	shandong	2011	iss	"NOR,ENR,TE,PME,DOX,EFT,AC"	10	0027
SX09-3	shanxi	2009	iucD	"ENR,GM,SXT,TE,SF,NOR"	354	0028
SD08-11	shandong	2008	"iucD,iss"	"AM,EFT,GM,SPT,TE,FFC,SF,SXT,ENR"	93	0029
SD11-3	shandong	2011	"papC,tsh,iss"	"NOR,ENR,TE,DOX,ETT,FFC,SF,SXT,AC"	354	0030
SD08-1	shandong	2008	"papC,lrp2,iss"	"NOR,ENR,TE,PME,GM,DOX,EFT,FFC,SF,SXT,AM"	2309	0031
SD08-8	shandong	2008	"iucD,iss,tsh,cva"	"AM,EFT,GM,SPT,TE,FFC,SF,SXT,ENR"	362	0032
SD12-1	shandong	2012	iss	"NOR,ENR,TE,GM,SPT,DOX,EFT,SXT,AC,AM"	165	0033
NM10-5	neimeng	2010	"iucD,iss"	"NOR,ENR,TE,PME,GM,DOX,EFT,FFC,SF,SXT,AC,AM"	2732	0034
SD08-6	shandong	2008	"iucD,iss"	"AM,EFT,GM,SPT,TE,FFC,SF,SXT,ENR"	2732	0035
AH08-6	anhui	2008	"iucD,lrp2"	"ENR,TE,GM,SPT,FFC,SF,SXT,AM"	93	0036
HN08-1	henan	2008	iss	"SPT,SF,SXT"	1101	0037
CQ11-3	chongqing	2011	"iucD,lrp2"	"NOR,ENR,TE,PME,GM,DOX,FFC,SF,SXT,AC,AM"	115	0038
SX10-4	shanxi	2010	"iucD,lrp2"	"NOR,ENR,TE,PME,GM,SPT,DOX,EFT,FFC,SF,SXT,AC,AM"	115	0039
CQ11-1	chongqing	2011	iucD	"NOR,ENR,TE,PME,GM,DOX,FFC,SF,SXT,AC"	115	0040
SD11-11	shandong	2011	"iss,vat"	"PME,GM,SPT,DOX,EFT,SXT,AC"	117	0041
SD11-9	shandong	2011	"iucD,iss,vat"	"ENR,TE,PME,SPT,EFT,FFC,SF,SXT,AC"	117	0041
SD11-6	shandong	2011	"iucD,iss,vat"	"PME,GM,SPT,EFT,SXT,AC"	117	0042
SD08-7	shandong	2008	"iucD,iss"	"AM,EFT,GM,SPT,TE,FFC,SF,SXT,ENR"	2732	0043
SX10-5	shanxi	2010	"iucD,lrp2"	"NOR,ENR,TE,EFT,FFC,SF,SXT,AC,AM"	2309	0044
SX09-5	shanxi	2009	"papC,iucD"	"ENR,AM,SXT,TE,SF,FFC,NOR"	2309	0045
NM10-2	neimeng	2010	"iucD,lrp2,tsh"	"ENR,TE,SPT,DOX,SF,SXT,AC"	2176	0046
SD11-1	shandong	2011	"iucD,lrp2,vat"	"PME,GM,SPT,DOX,EFT,SF,SXT,AC"	117	0046
SX10-6	shanxi	2010	"iucD,lrp2,iss,tsh"	"NOR,ENR,TE,PME,GM,SPT,DOX,EFT,FFC,SF,SXT,AC,AM"	88	0047
SD00-3	shandong	2000	"iucD,iss,tsh"	"NOR,TE,DOX,SF,SXT,AM"	101	0048
SX09-1	shanxi	2009	"iucD,lrp2"	"NOR,ENR,TE,GM,DOX,EFT,FFC,SF,SXT"	533	0049
HN08-4	henan	2008	"iucD,iss"	"ENR,SXT"	2165	0050
NM10-10	neimeng	2010	"iucD,lrp2,iss"	"TE,DOX,SF,SXT"	117	0051
CQ11-5	chongqing	2011	"iucD,tsh,iss"	"NOR,ENR,TE,PME,SPT,EFT,FFC,SF,SXT,AC,AM"	155	0052
SX10-7	shanxi	2010	"iucD,cva"	"NOR,ENR,TE,GM,SPT,DOX,EFT,FFC,SF,SXT,AC,AM"	3714	0053
SD08-12	shandong	2008	iucD	"AM,TE,FFC,SF,SXT,ENR"	3285	0054
SD08-3	shandong	2008	iucD	"AM,EFT,GM,TE,FFC,SF,SXT"	3285	0054
HN08-2	henan	2008	iss	"ENR,PME,SXT"	162	0055
SD11-10	shandong	2011	"tsh,cva"	"NOR,ENR,TE,PME,GM,DOX,EFT,AC"	453	0056
SD11-2	shandong	2011	"iucD,tsh,cva,iss"	"NOR,ENR,TE,GM,SPT,DOX,EFT,AC"	453	0057
SD08-13	shandong	2008	"iucD,lrp2,iss,cva"	"ENR,TE,GM,SPT,EFT,FFC,SF,SXT,AC,AM"	131	0058
SD08-14	shandong	2008	"iucD,lrp2,iss,cva"	"TE,SPT,EFT,FFC,SF,SXT,AC,AM"	131	0058
SD08-5	shandong	2008	"iucD,lrp2,iss,cva"	"AM,EFT,GM,SPT,TE,FFC,SF,SXT"	131	0058
SX10-8	shanxi	2010	"iucD,tsh"	"NOR,ENR,SPT,,EFT,FFC,SF,SXT,AC,AM"	354	0059
AH08-10	anhui	2008	"iucD,iss"	"ENR,TE,GM,EFT,FFC,SF,SXT,AC,AM"	115	0060
SD08-4	shandong	2008	lrp2	"AM,EFT,SPT,TE,FFC,SF,SXT,ENR"	189	0061
SX09-4	shanxi	2009	iucD	"ENR,AM,GM,SXT,TE,SF,FFC"	746	0062
AH08-1	anhui	2008	"iucD,lrp2"	"TE,SF,SXT"	48	0063
HN08-3	henan	2008	"iucD,iss,vat"	"ENR,TE,AM,SPT,EFT,SF,SXT,FFC"	1431	0064
SD12-3	shandong	2012	iss	"NOR,ENR,TE,DOX,EFT,SF,SXT,AC,AM"	10	0065
AH08-11	anhui	2008	"iucD,iss"	"TE,GM,FFC,SF,SXT,AM"	1158	0066
SX09-2	shanxi	2009	iucD	"NOR,ENR,DOX,EFTSF,SXT"	155	0067
SX10-1	shanxi	2010	"papC,iucD,lrp2"	"NOR,ENR,TE,GM,SPT,EFT,FFC,SF,SXT,AC,AM"	770	0068
HN08-6	henan	2008	"iucD,lrp2"	"ENR,EFT,SXT"	770	0069
SD11-8	shandong	2011	"papC,iucD"	"GM,SPT,EFT,SXT,AC"	1140	0070
AH08-3	anhui	2008	"lrp2,tsh"	"TE,FFC,SF,SXT"	1079	0071
AH08-9	anhui	2008	"iucD,iss"	"TE,SF,SXT"	1079	0071
NM10-1	neimeng	2010	tsh	"TE,DOX"	871	0072
NM10-7	neimeng	2010	"papC,tsh"	"TE,SPT,SF,SXT,AC,AM"	871	0072
CQ11-4	chongqing	2011	"iucD,lrp2,iss"	"NOR,ENR,TE,PME,GM,EFT,FFC,SF,SXT,AC,AM"	1551	0073
SD08-10	shandong	2008	iucD	"AM,EFT,GM,SPT,TE,FFC,SF,SXT,ENR"	354	0074
SD11-4	shandong	2011	"papC,tsh"	"NOR,ENR,TE,GM,DOX,STX"	354	0075

Figure 2. Dendrogram of PFGE profiles of 87 *E. coli* isolates from poutry frams PFGE patterns and the corresponding dendrogram for 87 isolates obtained in the present study are depicted. The 6 PFGE clusters were marked on the node as A to F. The different clusters observed are designated on the left side of the figure. Displayed on the right hand side are key (strain name), province, year, PFGE-Pattern, virulence gene, sequence type (ST) and antibiotic resistance. Abbreviations for antibiotics are: AM, Ampicillin, AC, Auge door-keeper, PME, Polymyxin, EFT, Cefotaxime, GM, Gentamicin, SPT, Spectinomycin, TE, Tetracycline, DOX, Doxycycline, SF, Sulfisoxazole, SXT, Trimethoprim-sulfamethoxazole, NOR, Ofloxacin, ENR, Enrofloxacin, FFC, Florfenicol.

ST23 isolates, including *iucD*, *Irp2*, *iss*, *cva*, *tsh* genes and other 3 ST23 isolates exhibit less one. The virulence profiles correspond inconsistently with PFGE type, but they gathered in a cluster, suggesting similar evolution of virulence genotypes. Meanwhile, the ST131 isolates exhibit the same virulence profiles and PFGE type.

Antibiotic resistance

All *E. coli* isolates were highly resistant to trimethoprim-sulfamethoxazole (SXT), tetracycline (TE) (85.06%), sulfisoxazole (SF) (83.91%), ampicillin (AM) (66.67%), fluoroquinolones [enrofloxacin (ENR), 63.22%, ofloxacin (NOR), 50.57%] and gentamicin (GM) (57 47%) (**Figure 1**). All isolates were multi-drug resistant as they were resistant to at least 2 groups of antimicrobials. Advantage of resistant performance is NOR-TE-DOX-SF-SXT-AM-GM (**Figure 2**).

All 87 *E. coli* isolates were analyzed by PFGE using enzymes *XbaI* resulted in 75 distinguishable patterns demonstrating a high level of genetic diversity among the isolates. An UPGMA dendrogram was constructed (**Figure 2**). Fifteen *E. coli* isolates were untypeable by PFGE. After the addition of thiourea to the running buffer, all isolates remained typeable. The 87 isolates could be divided into six clusters A to F at a similarity of 60% or greater. Cluster A contains AH08-8 (ST10) and NM10-11 (ST93). Most of isolates belongs to cluster B, in which including all ST23, ST77, ST2309 and ST2505 isolated, There were a variety of PFGE types for *E. coli* strains, but these were not very similar.

MLST typing

Thirty-nine discrete STs were identified among the 87 *E. coli* isolates, indicating a high degree of genotypic diversity. Of these 39 STs, 20 were represented by single isolates, 19 were represented by more than one isolate (n=2 to 10). The predominant STs were ST23 and ST354 containing 10 (256%) and 6 (154%) isolates respectively (**Table 2**). Isolates characterized as the same ST did not necessarily have the same PFGE pattern. For example, the 10 isolates characterized as ST23 had 8 distinct PFGE patterns (**Figure 2**). All of the isolates that shared a PFGE pattern had the same ST. Meanwhile, isolates of the same STs generally showed the same or similar drug resistance patterns (**Figure 2**). ST23, ST117, ST113 and ST2732 isolates showed the same or similar multi-drug resistance to 13 antimicrobial agents respectively.

A minimum spanning tree was constructed (**Figure 3**). Most STs differed from each other by 2 or more alleles while four pairs of STs (ST155 and ST3714, ST162 and ST2176, ST10 and ST48, ST165 and ST189 and ST115 and ST2309) and one set of 3 STs (ST23, ST88 and ST2505) differed from each other by only 1 allele.

Discussion

This study provided molecular-epidemiological data on *E. coli* strains isolated in the 32 intensive poultry farms in 6 provinces (Shandong, Shanxi, Neimeng, Anhui, Henan and Chongqing) in China from 2000 to 2012.

We analyzed multiple colonies from 39 samples to determine diversity within a sample (**Figure 2**). Two samples contained isolates with identical properties, suggesting they were the same strain, while the majority of the samples contained isolates belonging to the same sequence type but differing by one or more of the phenotypic or genetic properties tested, indicating that they were variants of the same clone. Most common variations were non-expression of the H antigen, variation of antibiotic resistance and/or variation in PFGE patterns.

Many studies have underlined the potential key role of the HIP subtypes in the severity of dis-

Table 2. ST and allele profile of each isolate

ST	adk	fumC	gyrB	icd	mdh	purA	recA	No of isolates
10	10	11	4	8	8	8	2	4
23	6	4	12	1	20	13	7	10
48	6	11	4	8	8	8	2	1
88	6	4	12	1	20	12	7	1
93	6	11	4	10	20	8	6	4
101	43	41	15	18	11	7	6	1
115	4	26	39	25	5	31	19	5
117	20	45	41	43	5	32	2	5
131	53	40	47	13	36	28	29	3
155	6	4	14	16	24	8	14	2
156	6	29	32	16	11	8	44	1
162	9	65	5	1	9	13	6	1
165	10	27	5	10	12	8	2	1
189	10	27	5	10	12	8	49	1
354	85	88	78	29	59	58	62	6
362	62	100	17	31	5	5	4	1
453	99	6	33	33	24	8	7	2
533	6	4	5	18	11	8	14	1
539	6	19	57	18	9	13	6	1
602	6	19	33	26	11	8	6	2
711	9	6	15	131	24	7	7	3
746	10	7	4	8	12	8	2	1
770	52	116	55	101	113	40	38	2
871	64	7	1	8	8	8	6	2
1079	6	19	14	16	11	12	2	2
1101	9	8	5	1	9	8	7	1
1125	6	4	15	18	24	26	7	1
1140	83	23	164	181	80	1	42	1
1158	18	3	17	6	5	5	4	1
1431	6	65	3	1	11	13	6	1
1551	6	250	83	28	1	1	2	1
1724	9	29	12	26	11	8	7	1
2165	6	23	3	16	9	7	7	2
2176	9	65	5	1	9	13	58	1
2309	271	26	39	25	5	31	19	4
2505	6	41	12	1	20	13	7	4
2732	46	26	208	6	5	16	4	3
3285	6	6	15	10	20	23	6	2
3714	6	4	14	402	24	8	14	1

ease. *Irp2* gene was the HPI core part, Zhu [23] proved that HPI already existed in poultry *E. coli* and the frequency was 17.1%. In this study, the prevalence of *Irp2* was 43.7%. Fimbrial adhesins play an important role in colonization of the chicken intestine, *papC* gene encodes the adherence factor intimin, in the study *papC* gene the frequency is low at 9.2%. Other virulence factors may contribute to the pathogenicity of *E. coli*, *iucD* gene was the most prevalent virulent gene (74 7%) among the 7 virulence genes in our text.

Many non-O157 STEC isolated from humans and animals have shown resistance to multiple antimicrobials including resistance to trimethoprim-sulfamethoxazole [24-27]. In our study, we found that only 1 of the 8 categories of antimicrobial resistance types (sulfonamides) and 2 of the 13 antimicrobial agents (sulfisoxazole (SF) and trimethoprim-sulfamethoxazole (SXT)) were active against most the isolates. The high prevalence (>50%) of resistance to tetracycline trimethoprim-sulfamethoxazole is similar to that of other studies in China [25, 27]. This suggests that the poultry farms in those countries may have used the similar antimicrobials for prophylactics as the poultry farms in China. PFGE, which is known for its discriminatory power as a molecular typing tool in epidemiologic studies, these isolates which separation in 2000 also shared identical ST and antimicrobial resistance profiles (**Figure 2**). All the isolates were obtained from different chicken raised in the same region, strongly suggesting that the transmission of the *E. coli* clone among the animals have occurred. In addition, the strains with the same PFGE type, the ST and resistant genes are the same, the opposite is not established, this results was consistent with Yu [28] studied, thus it can be seen that the same strains exist in the process of proliferation spread of resistant plasmids of gain or loss, lead to the change of the resistance, make have different resistant strains of the same type PFGE spectrum.

In this study, the variation of PFGE genotypes for *E. coli* is small and the overall similarity value is about 60%~100%, 87 *E. coli* isolates are divided into 75 PFGE typing, 39 subtypes, in which, 13 *E. coli* isolates from 2000 are divided into 8 PFGE typing, 3 subtypes, besides SD00-3, most of the strains are gathered in a cluster, although it is believed that these strains may be from the same clone, Dai [29] studied on 16 strains from different farms multi-resistant source of chicken *E. coli* PFGE classification analysis, 16 different belt type, which fully

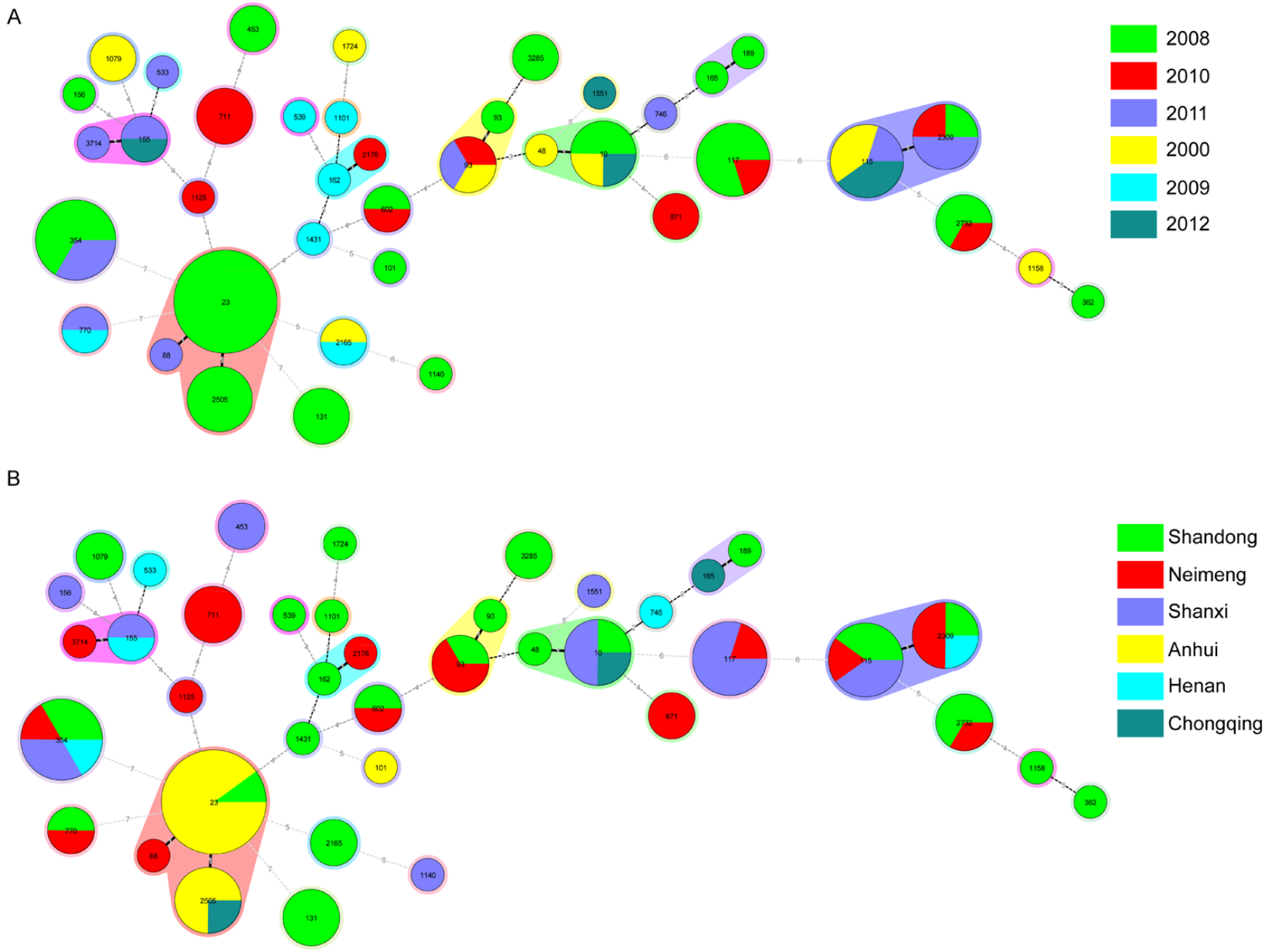

A
2008
2010
2011
2000
2009
2012
B
Shandong
Neimeng
Shanxi
Anhui
Henan
Chongqing

Figure 3. Genetic relationships of *E. coli* isolates based on MLST. A. The separation of area to build the genetic relationship of all isolates STs. The colors for the slices of the pie represent places of isolates: Shandong province in green, Neimeng province in red, Shanxi province in purple, Anhui province in yellow, Henan province in wathet and Chongqing city in reseda. The numbers on connecting lines show the number of allelic difference between two STs. The number in a circle is the ST number; B. The separation of time to build the genetic relationship of all isolates STs. Each circle represents a given ST with size proportional to the number of isolates. The number in a circle is the ST number. The colors for the slices of the pie represent places of isolates: Red for 2010, green for 2008, purple for 2011, 2000 in yellow, 2009 in bule and 2012 in reseda. The numbers on connecting lines show the number of allelic difference between two STs.

embodies the polymorphism distribution of *E. coli.*

All isolates of mapping results show PFGE and correlated with MLST, the strains have the same ST sequences, they are not necessarily the same PFGE typing, but the PFGE type with consistent. ST sequence must be the same, such as ST23 isolates, they come from the 2000 and 2008 different time and region, the PFGE typing are different. SD08-5. SD08-13 and SD08-14 they consistent with PFGE typing, the ST series are ST131. This relationship shows that *E. coli* between horizontal distribution and vertical transmission of isolates thus combining PFGE and MLST classification method is helpful to find popular advantage of molecular characteristics determine the region characteristics of the strain.

This study foud there was no obvious input or appearance of heterologous strains in the epidemic strains from 2000 to 2012 in China. The relationship between *E. coli* strains in China and *E. coli* strains in other regions around the country need further studies. Some of other countries and regions have established their own PulseNet [19]. The results of this study provide PFGE fingerprints of *E. coli* strains in China and establish a good foundation for the realization of data sharing, which will help to realize active surveillance of *E. coli* disease and tracing the source of infection in China.

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Disclosure of conflict of interest

None.

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