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Predominance of $bla_{CTX\text{-}M\text{-}65}$ and $bla_{CTX\text{-}M\text{-}55}$ in extended-spectrum β-lactamase-producing *Escherichia coli* from raw retail chicken in South Korea

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ABSTRACT

Objectives: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) are a serious public health concern worldwide. The aim of this study was to characterise ESBL-EC isolated from raw retail chicken in South Korea.
Methods: The antimicrobial resistance, phylogenetic group and virulence gene prevalence of 67 ESBL-EC isolated from retail chicken in South Korea were investigated.
Results: All of the isolates possessed $bla_{CTX\text{-}M}$ genes, predominantly $bla_{CTX\text{-}M\text{-}65}$ (52.2%) and $bla_{CTX\text{-}M\text{-}55}$ (25.4%), and three isolates harboured both $bla_{CTX\text{-}M\text{-}65}$ and $bla_{CTX\text{-}M\text{-}55}$. More than one-half of the ESBL-EC strains also carried bla_{TEM}. Antimicrobial susceptibility testing revealed that 98.5% of the strains were multidrug-resistant (MDR). Phylogenetic analysis showed that group A was predominant (56.7%), followed by B1 (19.4%), E (8.9%), B2 (6.0%) and D (6.0%). Virulence genes associated with extraintestinal pathogenic *E. coli* (ExPEC) were frequently detected in isolates of phylogenetic groups B1, B2, D and E.
Conclusion: The results in this study demonstrate that retail chicken in South Korea is highly contaminated with MDR ESBL-EC and may serve as a reservoir for transmitting ExPEC to humans.

1. Introduction

Extended-spectrum β-lactamases (ESBLs) are a group of enzymes that hydrolyse most β-lactams, including penicillins, cephalosporins and monobactams, but not carbapenems [1]. Since ESBL genes are typically encoded on mobile genetic elements, usually plasmids, these genes may be easily disseminated [2]. The mobility of ESBL genes has resulted in a rapid increase in the prevalence of ESBL-producing Enterobacteriaceae in food-producing and companion animals, environmental samples (e.g. wastewater) and even food [3]. ESBL-producing *Escherichia coli* (ESBL-EC) are frequently isolated from chicken meat [4–8]. In some cases, the high prevalence of ESBL-EC hampers the isolation of other fastidious bacteria (e.g. *Campylobacter*) from chicken as it outgrows during the enrichment step using cephalosporins as a selective supplement [9].

Pathogenic *E. coli* are a major cause of not only enteric diseases but also extraintestinal infections such as urinary tract infections [10]. Although extraintestinal pathogenic *E. coli* (ExPEC) cause infections outside the intestines, ExPEC first colonise the gastrointestinal tract and are transmitted primarily by the consumption of food, particularly chicken [11]. Commensal *E. coli* isolates usually harbour no or only a very few virulence genes; however, ExPEC possess a broad range of virulence genes involved in bacterial adhesion, iron acquisition and serum survival as well as toxins associated with extraintestinal disease [12]. ExPEC commonly possess large, transmissible, multidrug resistance plasmids encoding ESBLs [13], suggesting that chicken could be a source both for ExPEC and ESBL-EC.

A number of studies have shown that retail chicken is significantly involved in transmitting ESBL-EC and ExPEC to humans [4,13,14]. Despite its public health importance, the prevalence of ESBL-EC on retail chicken in South Korea has been reported in only a single study that characterised only a limited number ($n = 6$) of ESBL-EC from retail chicken [15]. Furthermore, there is no study regarding ESBL-EC from retail chicken, although they may have the potential to cause

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Table 2
Antimicrobial resistance patterns of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates ($n = 67$) according to ESBL gene type.

Antimicrobial agent	Resistance breakpoint (μg/mL)	No. (%) of resistant strains			
		CTX-M ($n = 29$)	CTX-M + TEM ($n = 32$)	CTX-M + TEM + OXA ($n = 6$)	Total ($n = 67$)
CIP	≥4	13 (44.8)	21 (65.6)	5 (83.3)	39 (58.2)
TET	≥16	26 (89.7)	23 (71.9)	6 (100)	55 (82.1)
CHL	≥32	19 (65.5)	25 (78.1)	6 (100)	50 (74.6)
KAN	≥64	6 (20.7)	8 (25.0)	4 (66.7)	18 (26.9)
GEN	≥16	12 (41.4)	17 (53.1)	3 (50.0)	32 (47.8)
STR	≥64	25 (86.2)	24 (75.0)	6 (100)	55 (82.1)
COL	≥8	2 (6.9)	0	0	2 (3.0)
AMP	≥32	29 (100)	32 (100)	6 (100)	67 (100)
CEF	≥32	29 (100)	32 (100)	6 (100)	67 (100)
CRO	≥4	29 (100)	32 (100)	6 (100)	67 (100)
CTX	≥4	29 (100)	32 (100)	6 (100)	67 (100)

CIP, ciprofloxacin; TET, tetracycline; CHL, chloramphenicol; KAN, kanamycin; GEN, gentamicin; STR, streptomycin; COL, colistin; AMP, ampicillin; CEF, cefalotin, CRO, ceftriaxone; CTX, cefotaxime.

extraintestinal infections in humans. To fill this important knowledge gap, in this study the antimicrobial resistance and virulence gene prevalence of ESBL-EC isolated from retail raw chicken in South Korea were characterised.

2. Materials and methods

2.1. Collection of extended-spectrum β-lactamase-producing E. coli from raw retail chicken in South Korea

A total of 67 ESBL-EC were isolated from 40 retail raw whole chicken samples from 28 companies in six different provinces of South Korea in our previous study (submitted). *E. coli* were grown on MacConkey agar and were confirmed by 16S rRNA sequencing (Macrogen, Seoul, South Korea). To confirm the ESBL phenotype, the *E. coli* isolates were subjected to the modified ESBL confirmatory test of the Clinical and Laboratory Standards Institute (CLSI), which uses antimicrobial disks of cefotaxime and ceftazidime with or without the ESBL inhibitor clavulanic acid, and boric acid and ethylene diamine tetra-acetic acid (EDTA) to inhibit AmpC β-lactamases and carbapenemases, respectively [16]. *E. coli* ATCC 25922, a CLSI quality control strain, was used as a negative control. The presence of ESBL genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M} and bla_{OXA}) was determined by PCR using previously described primers [17]. PCR amplicons were sequenced and the translated amino acid sequences were used to determine the ESBL gene type [18–20]. The *E. coli* isolates were routinely cultured on Luria–Bertani medium.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was determined by the broth dilution method using a total of 11 antibiotics, including ampicillin, cefalotin, tetracycline, chloramphenicol, kanamycin, gentamicin, streptomycin, colistin, ceftriaxone, cefotaxime and ciprofloxacin. Minimum inhibitory concentrations (MICs) were determined according to CLSI guidelines and previous reports [21–23]. *E. coli* ATCC 25922 was used as a quality control strain according to the CLSI protocol.

2.3. Phylogenetic analysis of extended-spectrum β-lactamase-producing E. coli

The phylogenetic group of the ESBL-EC isolates was determined using a quadruplex PCR-based method amplifying *chuA*, *yjaA*, DNA fragment *TspE4C2* and *arpA* [24]. *E. coli* strains MG1655 and ATCC 25922 were used as controls for phylogenetic groups A and B2, respectively.

2.4. Random amplified polymorphic DNA (RAPD) analysis

RAPD analysis was used as a PCR-based DNA fingerprinting method to analyse clonal similarity of the isolates. DNA extracted from the 67 *E. coli* isolates was subjected to PCR using previously reported primers [25] and the PCR results were analysed using BioNumerics v.7 software (Applied Maths, Sint-Martens-Latem, Belgium).

2.5. Detection of virulence genes associated with pathogenic E. coli

PCR was performed to analyse the presence of virulence genes associated with five major intestinal pathogenic *E. coli* groups, including Shiga toxin-producing *E. coli* (STEC) (*stx1* and *stx2* encoding Shiga toxins 1 and 2, *hylA* encoding enterohaemolysin and *espP* encoding serine protease), enteropathogenic *E. coli* (EPEC) (*eaeA* encoding intimin), enterotoxigenic *E. coli* (ETEC) (*st* and *lt* encoding heat-stable and heat-labile enterotoxins) and enteroaggregative *E. coli* (EAEC) (*aggR* encoding a transcription regulator for aggregative adherence fimbria I) and ExPEC [26,27]. The tested virulence genes related to ExPEC included extraintestinal *E. coli* attachment factors (*fimH* and *iha*), iron uptake factors (*aer*, *irp2* and *iutA*), iron transporter (*feoB*), increased serum survival protein (*iss*) and heat-stable enterotoxin (*astA*) [28–30]. *E. coli* ATCC 35150 was used as a positive control for STEC and EPEC. *E. coli* NCCT 14039 was a positive control for EAEC. *E. coli* MG1655 was used as a negative control for the tested virulence genes except for *fimH* and *feoB*. *E. coli* strains ATCC 43888 and O169 were used as positive controls for *iha* and *astA*, respectively. *E. coli* strains MG1655 and ATCC 25922

Table 1
Distribution of extended-spectrum β-lactamase (ESBL) types in ESBL-producing *Escherichia coli* isolates ($n = 67$) from raw retail chicken in South Korea.

ESBL group	ESBL type	No. of strains
CTX-M group 1	CTX-M-55	2
CTX-M group 1, TEM	CTX-M-55, TEM-1	6
	CTX-M-55, TEM-116	6
	CTX-M-15, TEM-1	1
	CTX-M-15, TEM-135	1
CTX-M group 9	CTX-M-65	17
	CTX-M-14	7
	CTX-M-27	1
CTX-M group 9, TEM	CTX-M-65, TEM-1	3
	CTX-M-65, TEM-116	7
	CTX-M-14, TEM-1	6
	CTX-M-14, TEM-116	2
Miscellaneous	CTX-M-55, CTX-M-65	2
	CTX-M-65, OXA-1, TEM-1	5
	CTX-M-55, CTX-M-65, OXA-1, TEM-1	1

Table 3
Phylogenetic group of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates ($n = 67$) from raw retail chicken in South Korea.

Phylogenetic group	No. (%) of strains
A	38 (56.7)
B1	13 (19.4)
B2	4 (6.0)
D	4 (6.0)
E	6 (9.0)
F	1 (1.5)
Unknown	1 (1.5)

were used as positive and negative controls, respectively, for *fimH* and *feoB*.

2.6. Statistical analysis

The statistical significance of the distribution of virulence genes was analysed by χ^2 test using GraphPad Prism software v.5 (GraphPad Software Inc., La Jolla, CA).

3. Results

3.1. Distribution of extended-spectrum β-lactamase genes in E. coli from raw retail chicken

All of the ESBL-EC strains from retail poultry possessed $bla_{\text{CTX-M}}$ (Table 1), suggesting that CTX-M is the predominant ESBL type in *E. coli* from retail chicken in South Korea. More than one-half (56.7%; 38/67) of the $bla_{\text{CTX-M}}$-positive strains also carried bla_{TEM} and/or bla_{OXA}, whereas bla_{SHV} was not detected (Table 1). The dominant CTX-M types included CTX-M-65 (52.2%; 35/67) in the CTX-M group 9 and CTX-M-55 (25.4%; 17/67) in the CTX-M group 1; three strains harboured both $bla_{\text{CTX-M-65}}$ and $bla_{\text{CTX-M-55}}$ (Table 1). The $bla_{\text{OXA-1}}$ gene was detected in six (9.0%) of the 67 ESBL-EC strains, and the $bla_{\text{OXA-1}}$-positive strains also harboured $bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M-65}}$ and/or $bla_{\text{CTX-M-55}}$ (Table 1).

3.2. Antimicrobial susceptibility of extended-spectrum β-lactamase-producing E. coli from raw retail chicken

Strains harbouring all of the $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{OXA} genes were highly resistant to all tested antibiotics except for colistin (Table 2). Antimicrobial susceptibility testing showed that 98.5% (66/67) of the tested ESBL-EC isolates were resistant to at least three antibiotic classes tested in this study, suggesting that ESBL-EC from retail chicken in South Korea is highly multidrug-resistant (MDR).

3.3. Phylogenetic group analysis of extended-spectrum β-lactamase-producing E. coli from raw retail chicken

The predominant phylogenetic groups of the ESBL-EC isolates from retail chicken were group A (56.7%) and group B1 (19.4%). Four *E. coli* isolates each belonged to groups B2 and D, respectively. Eight strains were classified as minor group, of which six belonged to group E, one to group F and one strain was unknown (Table 3). Phylogenetic analysis using RAPD-PCR showed that ESBL-EC isolates belonging to the same phylogenetic group tended to form the same cluster (Supplementary Fig. S1).

3.4. Prevalence of virulence genes in extended-spectrum β-lactamase-producing E. coli from raw retail chicken

The prevalence of virulence genes representing the five major pathogenic groups of *E. coli*, including ETEC, EPEC, EAEC, STEC and ExPEC, was examined. The *espP* genes was detected and in one strain in group B1 and the *eaeA* gene was detected five strains in group A, whereas toxin genes were not detected. Interestingly, all 67 ESBL-EC isolates carried at least one ExPEC-related virulence gene. Compared with group A, the prevalence of ExPEC-related virulence genes was more frequent in groups B1, B2, D and E (Table 4), and those harbouring at least six ExPEC-related virulence genes belonged to phylogenetic groups B1, B2, D and E with statistical significance (Table 5).

Table 4
Prevalence of virulence genes of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates according to phylogenetic group.

Pathogenetic group	Virulence gene	No. (%) of strains							
		Group A ($n = 38$)	Group B1 ($n = 13$)	Group B2 ($n = 4$)	Group D ($n = 4$)	Group E ($n = 6$)	Group F ($n = 1$)	Unknown ($n = 1$)	Total ($n = 67$)
ExPEC	*astA*	3 (7.9)	5 (38.5)*	1 (25.0)	4 (100)***	3 (50.0)**	0	0	16 (23.9)
	iss	27 (71.1)	9 (69.2)	3 (75.0)	2 (50.0)	6 (100)	1 (100)	1 (100)	49 (73.1)
	fimH	21 (55.3)	13 (100)**	4 (100)	4 (100)	6 (100)*	1 (100)	1 (100)	50 (74.6)
	aer	22 (57.9)	10 (76.9)	4 (100)	4 (100)	6 (100)*	1 (100)	1 (100)	48 (71.6)
	irp2	3 (7.9)	7 (53.8)***	3 (75.0)***	1 (25.0)	0	0	0	14 (20.9)
	iha	1 (2.6)	3 (23.1)*	0	0	0	0	0	4 (6.0)
	iutA	21 (55.3)	10 (76.9)	4 (100)	4 (100)	6 (100)*	1 (100)	1 (100)	47 (70.1)
	feoB	38 (100)	13 (100)	4 (100)	4 (100)	6 (100)	1 (100)	1 (100)	67 (100)
STEC	*stx1*	0	0	0	0	0	0	1 (100)	1 (100)
	stx2	0	0	0	0	0	0	0	0
	hylA	0	0	0	0	0	0	0	0
	espP	0	1 (7.7)	0	0	0	0	0	1 (1.5)
EPEC	*eaeA*	5 (13.2)	0	0	0	0	0	0	5 (7.5)
ETEC	*st*	0	0	0	0	0	0	0	0
	lt	0	0	0	0	0	0	0	0
EAEC	*aggR*	0	0	0	0	0	0	0	0

ExPEC, extraintestinal pathogenic *E. coli*; STEC, Shiga toxin-producing *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; EAEC, enteroaggregative *E. coli*.
* $P < 0.05$, distribution in indicated group versus group A.
** $P < 0.01$, distribution in indicated group versus group A.
*** $P < 0.001$, distribution in indicated group versus group A.

Table 5
Distribution of extended-spectrum β-lactamase-producing *Escherichia coli* isolates in phylogenetic groups according to the number of virulence genes.

Phylogenetic group	No. (%) of virulence genes						
	7	6	5	4	3	2	1
Group A (*n* = 38)	0	0	7 (18.4)	15 (39.5)	11 (28.9)	3 (7.9)	2 (5.3)
Group B1 (*n* = 13)	3 (23.1)**	5 (38.5)***	2 (15.4)	0**	3 (23.1)	0	0
Group B2 (*n* = 4)	1 (2.5)**	1 (2.5)**	2 (50)	0	0	0	0
Group D (*n* = 4)	1 (2.5)**	1 (2.5)**	2 (50)	0	0	0	0
Group E (*n* = 6)	0	3 (50)***	3 (50)	0	0	0	0
Group F (*n* = 1)	0	0	1 (100)*	0	0	0	0
Unknown (*n* = 1)	0	0	1 (100)*	0	0	0	0

* $P < 0.05$, distribution in indicated group versus group A.
** $P < 0.01$, distribution in indicated group versus group A.
*** $P < 0.001$, distribution in indicated group versus group A.

4. Discussion

ESBL-EC are frequently isolated from poultry [6], and retail chicken is considered as an important vehicle transmitting ESBL-EC to humans [5]. In this study, ESBL-EC isolates from retail chicken in South Korea were extensively characterised. Consistent with the global expansion of CTX-M ESBLs [31], CTX-M was the predominant ESBL type in *E. coli* strains from retail chicken in South Korea. In this study, $bla_{CTX\text{-}M\text{-}65}$ (52.2%), $bla_{CTX\text{-}M\text{-}55}$ (25.4%) and $bla_{CTX\text{-}M\text{-}14}$ (22.4%) were the most common ESBL genes. Similarly, $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}65}$ and $bla_{CTX\text{-}M\text{-}14}$ were commonly detected in ESBL-EC from chicken in China [32]. In Japan, $bla_{CTX\text{-}M\text{-}2}$, bla_{TEM} and $bla_{CTX\text{-}M\text{-}1}$ were present in 45%, 36% and 34% of ESBL-EC isolates from domestic retail chicken meat samples, respectively [7]. In the Netherlands, among 87 strains of ESBL-EC from chicken meat, 69% harboured $bla_{CTX\text{-}M\text{-}1}$ [33]. Similarly, 65.4% of chicken meat samples in the UK were contaminated with ESBL-EC, and $bla_{CTX\text{-}M\text{-}1}$ was predominant (82.7%) [34]. Based on the findings in this and other studies, ESBL-EC are highly prevalent on retail chicken in most countries, however the dominant ESBL gene types are different depending on the geographic region.

All of the ESBL-EC isolates from retail chicken in the current study were resistant to multiple drugs belonging to different classes, such as ampicillin, tetracycline, chloramphenicol and streptomycin (Table 2). A high prevalence of MDR ESBL-EC has been reported previously. In China, 96.9% of ESBL-EC isolated from chickens were resistant to at least three different antimicrobial classes [35]. MDR strains are highly distributed in clinical as well as chicken samples. For instance, all of the ESBL-EC isolated from hospitals in India were MDR [36]. In the current study, two strains were resistant to colistin, an antibiotic of last-resort (Table 2). Further investigation found that the strains were positive for *mcr-1* (data not shown), the plasmid-encoded gene conferring resistance to colistin [37]. This observation may be explained by the fact that ESBL genes are usually encoded on transmissible plasmids harbouring multiple resistance genes [38]. Strains carrying three different ESBL genes (i.e. $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{OXA}) exhibited increased antimicrobial resistance compared with those harbouring fewer ESBL genes, although this was not statistically significant owing to the small number of strains ($n = 6$) (Table 2). Presumably, the co-existence of different ESBL genes may result from the co-presence of multiple resistance plasmids in the same strain. For instance, it has been demonstrated that plasmid-mediated quinolone resistance genes are frequently detected in ESBL-EC isolates from poultry [39].

Virulent ExPEC usually belong to phylogenetic groups B2 and D [40,41]. However, in the current study ESBL-EC isolated from retail chicken mainly belonged to groups A (56.7%) and B1 (19.4%), and only four strains were assigned to each of groups B2 and D (Table 3). Interestingly, the number of strains in group E, which is classified as a minor group, was larger than those belonging to groups B2 and D (Table 3). *E. coli* O157:H7 EDL933 is the best-known member belonging to group E [24]. Consistently, a previous study in Jeonnam Province in South Korea showed that the majority of *E. coli* isolates from chicken were limited to phylogenetic group A, followed by group B1, and no isolate belonged to group B2 [42]. A similar pattern of distribution was also found among isolates from chicken carcasses in China [43]. In contrast, *E. coli* isolates from chicken meat had the greatest percentage of group B1 strains (44%), followed by groups A (28%) and D (23%) in the Netherland [33]. Among *E. coli* isolates from chicken meat in the USA, groups D, B2, A and B1 were dominant in that order [44]. Thus, the distribution of phylogenetic groups may be affected by their geographical region. However, the prevalence of strains belonging to group E in the current study was similar to a previous report from the Netherland [33]. Whereas the isolates harboured no or only very few virulence factors related to gastrointestinal infection, various virulence factors associated with ExPEC were frequently detected in ESBL-EC isolates, particularly in those belonging to groups B1, B2, D and E, but not to group A (Tables 4 and 5). These results suggest that ESBL-EC isolates in groups B1, B2, D and E from retail chicken may potentially be implicated in extraintestinal infections in humans in South Korea.

The findings of this study demonstrate that raw retail chicken in South Korea could be a major source of disseminating ESBL-EC with great potential to cause extraintestinal infections. Since ESBL-EC from chicken are often MDR, further investigation is required to control and reduce the contamination of chicken meat by ESBL-EC to protect public health.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jgar.2019.01.005.

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