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Phenotypic characteristics and antimicrobial susceptibility of verotoxin-producing *E coli* from slaughtered cattle

Jae-Won Byun, Kyoung-Ho Kim[1], Sung-Mo Lee,
Hyun-Soon Hwang, Yong-Hee Kim

[1]*Veterinary faculty of Incheon Metropolitan Public Health & Environmental Research Institute, Incheon, 404-812, Korea*
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Abstract

Ten isolates of Verotoxin-producing *Escherichia coli* (VTEC) were detected in slaughtered cattle and investigated their phenotypic characteristics and antimicrobial susceptibility. None of the isolates was positive for *eae* gene. Only one isolate was positive for *uid*A gene. Eight out of ten isolates of VTEC were originated from broker's cattle. Thus microbiological monitoring for broker farms should be performed to minimize VTEC contamination. In the antimicrobial susceptibility test, all the isolates were highly resistant to bacitracin and lincomycin whilst they are susceptible to apramycin and neomycin.

Key words : VTEC, Antimicrobial susceptibility test, Slaughtered cattle

Introduction

Verotoxin-producing *Escherichia coli* (VT : Shiga-like toxin) is responsible for the human gastrointestinal disorders such as diarrhea and haemolytic colitis (HC), which was firstly reported from a patient ingesting uncooked ground beef in USA[1)]. Although *E. coli* O157:H7 is the best known Shiga-like toxin producing *E coli* (STEC), non-O157 STEC serotypes (eg, O111, O26) have caused haemolytic colitis in human[2-4)]. The most frequently implicated vector for O157-related *E coli* outbreaks is associated with ground beef and bovine-derived products have been linked to approximately 75% of outbreaks[5)]. It has been widely accepted that ruminants are natural reservoirs of STEC. Approximately, 30% of all cattle could be

[1]Corresponding author
Phone : +82-32-572-3145 Fax : +82-32-576-7785
E-mail : kimdocter@hanmail.net

a carrier animal.

There are several virulence factors produced by STEC such as verotoxin (VT), intimin, haemolysin, iron transport protein and lipopolysaccharide[3, 6, 7]. Among these factors, intimin is a 97 kDa outer membrane protein to adhere on the intestinal mucosa and was associated with an attaching and effacing lesion. The *eae* gene was homologous in human isolates but less in cattle. Accordingly, *eae* gene could be better indicator for the pathogenic STEC than the strain only producing verotoxin. A slaughter house is the first step of bovine food production and infectious agents control. Therefore, data on the prevalence and concentration of STEC in slaughtered animals are critical to control pathogens via a critical point control program. The aims of study were to determine the incidence of STEC in slaughtered cattle and survey their antimicrobial resistance.

Materials and Methods

Isolation of STEC

This study was carried out from March to November in 2004 at a slaughter house implicating HACCP located in Incheon city. Two hundred and eighty samples of bovine feces were examined by a modified Fukushima's method[8, 9]. Briefly, ten grams of stool samples were treated with 0.125N HCl-0.5% NaCl solution (20 ㎖) and incubated for 2 hours at 37℃ after homogenizing for 1-2 min. Then, 20 ㎕ of suspension was plated onto sorbitol MacConkey (sMaC) agar (Difco, USA) using a sterile glass stick. The colony of pink and colorless was transferred onto eosin methylene blue (EMB) agar (Merck, Germany). The distinctive colonies of *E coli* showing metallic green sheen were selected and stored at -70℃ for further study. API 20E strip (Biomerioux, France) was used to compare the carbohydrate fermentation and biochemical characteristics of the isolates with positive control *E coli* O157:H7 strain.

PCR assay for VT and minimal inhibition concentration (MIC) determination

Multiplex PCR method[10] was carried out to detect an ability of producing Shiga-like toxin (VT Ⅰ, VT Ⅱ), intimin (*eae*) and *uid*A. Briefly, the isolates were cultured in mEC broth (Difco, USA) for 18 hrs at 37℃ and centrifuged for 5 min at 7,000 rpm. The sediment was suspended with TE buffer and boiled for 10 min. Following a centrifugation, the supernatant was used as templates. The oligonucleotide primers were listed in Table 1. The samples were amplified for 35 cycles and each cycle consisted of 1 min at 94℃, 1 min at 60℃ and 1 min at 72℃. The PCR products were separated by electrophoresis in 1% agarose gels and stained with ethidium bromide. MIC was determined by a micro-dilution method with Muller-Hinton broth using sterile 96 well plates. The MIC was determined with the lowest concentration of antimicrobial agent that suppresses visible bacterial growth. Reference strain of *E coli* ATCC 25922 was included as an internal control. The antimicrobial agents were selected as follows: apramycin, neomycin, chlorotetracycline, oxytetracycline, tylosin, cephalothin, nalidixic acid and sulfadiazine.

Table 1. The oligonucleotide sequence of primers

Primers	Nucleotide sequence	Target	PCR product (bp)
LP30	5′-GAGTTAATGTGGTGGCGAAGG-3′	VT I	348
LP31	5′-CACCAGACAATGTAACCGCTG-3′		
LP43	5′-ATCCTATTCCCGGGAGTTTACG-3′	VT II	584
LP44	5′-GCGTCATCGTATACACAGGAGC-3′		
PT2	5′-GCGAAAACTGTGGAATTGGG-3′	*uid* A	252
PT3	5′-TGATGCTCCATCACTTCCTG-3′		
AE19	5′-CAGGTCGTCGTGTCTGCTAAA-3′	*eae* A	1,087
AE20	5′-TCAGCGTGGTTGGATCAACCT-3′		

Results

We examined biochemical properties and genotypes of *E coli* isolated from 280 cattle feces. Firstly, the feces were screened by acid treatment and PCR assay for the detection of VT toxin. Ten isolates (3.6%) out of 280 isolates were shown VT gene in PCR test (Table 2). One isolate was negative for sorbitol fermentation and has a uidA gene. Nine of 10 isolates contained VT I gene. No isolate was positive for eaeA gene. Eight of 10 isolates were originated from broker farm cattle. Minimal inhibition concentration (MIC) of isolates was determined by a broth dilution method (Table 3). The isolates were highly resistant to bacitracin and lincomycin, but were susceptible to apramycin and neomycin.

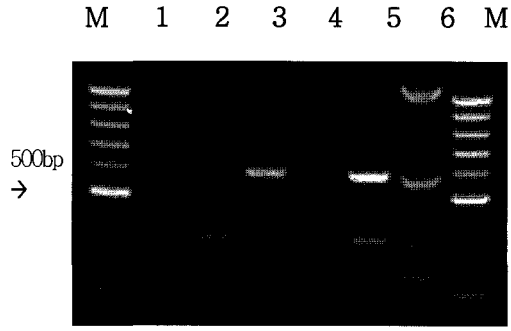

Fig 1. Result of agarose gel electrophoresis. Lane M : 100 bp ladder, Lane 1-5 : Isolates obtained from slaughtered cattle, Lane 6 : *E coli* 0157:H7

Table 2. Phenotypic characteristics of STEC isolates from slaughtered cattle

Strains	Acid tolerance	Sorbitol fermentation	PCR gene				Origin of cattle
			uidA	VT I	VT II	*eaeA*	
IC0401	+	+	-	-	+	-	farm
IC0405	+	-	+	+	-	-	〃
IC0407	+	+	-	+	-	-	broker
IC0408	+	+	-	+	-	-	〃
IC0409	+	+	-	+	-	-	〃
IC04010	+	+	-	+	-	-	〃
IC04011	+	+	-	+	+	-	〃
IC04012	+	+	-	+	-	-	〃
IC04013	+	+	-	+	+	-	〃
IC04014	+	+	-	+	+	-	〃

Table 3. MIC determination of the VTEC isolates from slaughtered cattle

Strains	Antimicrobials (μg/mg)							
	APR*	BA	LINCO	NEO	CTC	OTC	SUL	TYL
IC0401	10	1280	1280	2.5	160	1280	640	640
IC0405	5	640	1280	5	2.5	80	80	1280
IC0407	2.5	160	80	5	2.5	5	40	80
IC0408	10	640	1280	2.5	2.5	2.5	40	320
IC0409	5	640	1280	2.5	2.5	2.5	40	320
IC04010	10	640	1280	2.5	2.5	2.5	80	320
IC04011	5	640	640	2.5	2.5	20	20	160
IC04012	2.5	320	640	2.5	2.5	2.5	640	160
IC04013	2.5	640	640	2.5	2.5	2.5	1280	160
IC04014	2.5	640	640	2.5	10	40	1280	320

*: APR; apramycin, BA; bacitracin , LINCO; lincomycin, NEO; neomycin, CTC; chlorotetracycline, OTC; oxytetracycline, SUL; sulfadiazine, TYL; tylosin.

Discussion

VTEC O157:H7 food poisoning in human has been associated with contaminated beef products. Cattle have been identified as a principle reservoir of O157 and other VTEC. In this study, we determined the incidence of VTEC in slaughtered cattle and survey antimicrobial resistance. We employed an acid-shock step to foster the ability of selection and detection of VTEC. This method confirmed that *E coli* including VTEC has resistance to HCl and HCl treatment is effective for selection of *E coli* from other Gram-negative bacteria[8,9]. In this study, all VT-positive organisms isolated from cattle were identified as *E coli* on the basis of biochemical properties. Therefore, VT-PCR positive fecal samples were defined as VTEC positive.

Ten isolates (3.6%) out of 280 cattle were positive. Although all the isolates recovered in our study were not *E coli* O157:H7, the positive rate of VT-producing *E coli* was slightly higher than that of Kim et al[11] but less than that of Jung et al[10]. Moreover recent reports revealed that the rate of *E coli* O157:H7 infection in slaughtered cattle represented 1.3-28% in USA, 16.1%-16.6% in Italy, 10.6% in Netherland, 4.0%-4.7% in England, 0.2% in France and 1.6-8.1% in Japan[2,9]. It is also known that VTEC incidence is seasonal[12].

The possible causes of low incidence in this study were that the pattern of shedding VTEC is affected by diet, age and seasonal condition. Eight of 10 isolates recovered in this study were originated from broker's cattle. These results support that the broker farms have been consistently contaminated with feces containing VTEC shed from previously

infected cattle. Bryne et al[13] reported that downer cattle have much higher prevalence of VTEC than healthy cattle. In many cases brokers purchase downer or unhealthy cattle and raise them in their farms in Korea. Therefore, monitoring for these farms should be considered to minimize VTEC contamination.

In antimicrobial susceptibility test, all the selected antimicrobial reagents have been used as feed additives in the field. Nine of 10 isolates were resistant to bacitracin and lincomycin, but susceptible to apramycin, neomycin, chlorotetracycline and oxytetracycline. Sulfadiazine and tylosin show intermediate resistance. Studies about antibiotic resistance in USA showed that all isolates were resistant to tilmicosin and most isolates were susceptible to trimethoprim/sulfamethoxazole and ciprofloxacin[14, 15]. In Japan[16] antibiotic resistant patterns were observed to ampicillin, fosfomycin, kanamycin and vancomycin. Our study shows that the prevalence of VTEC was much lower than that of other countries. However, VTEC contamination in slaughter processing could have a possibility to cause food poisoning.

Therefore, more strict HACCP program should be applied to slaughter houses and meat processing plants. Continuous monitoring and surveillance program for microbial contamination at the slaughtering level should be performed to minimize the risk of outbreak of foodborne pathogens. Further studies are required to determine O and H antigen and to confirm O157 or non-O157(O111, O26) VTEC via cytotoxic test in vero cell.

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