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Characterization of class 1 integrons-mediated antibiotic resistance among calf pathogenic *Escherichia coli*

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Abstract

Escherichia coli isolates from calf diarrhea cases ($n = 22$) in the Beijing surrounding region in China were characterized for disease serotype, virulence factors, antimicrobial susceptibility pattern and class 1 integrons. 59% ($n = 13$) of the isolates were positive for the int I1 gene. The presence and genetic content of class 1 integrons in 13 *E. coli* isolates were examined by PCR and sequencing. Sequencing analysis revealed six gene cassettes, which encoded resistance to trimethoprim (*dfrA1*, *dfrA17*), aminoglycosides (*aadB*, *aadA1* and *aadA5*) and chloramphenicol (*cmlA*). The gene cassette arrays dfrA1–orf (45%) and aadB–orf–cmlA (32%) were most prevalent among these isolates. These data revealed the high prevalence of class 1 integrons among calf pathogenic *E. coli* isolates in the Beijing surrounding region in China, which may provide important and useful surveillance information reflecting specific antibiotic selective pressure.

Keywords: *Escherichia coli*; Integron; Gene cassette; Antibiotic resistance

1. Introduction

Calf diarrhea caused by colibacillosis, which is severe and costly, is becoming an increasingly challenging disease in animal production. *Escherichia coli* is considered as the most important bacterial cause, especially the enterotoxigenic *E. coli* [1]. One of the measures taken in treating *E. coli*-related colibacillosis is antimicrobial therapy. However, the broad use of antimicrobials in animal production may lead to the emergence and dissemination of antimicrobial-resistant bacterial pathogens, which without doubt pose a potential threat to human public health.

In recent years, a novel system known as integrons, which are mobile DNA elements with a specific structure consisting of two conserved segments flanking a central region containing unknown open reading frames or ''cassettes'' encoding specific antimicrobial resistance, has been identified in multiple resistant bacteria and is considered to play an important role in the acquisition and dissemination of antibiotic resistance genes [2,3]. To date, four classes of integrons have been identified [4–6] and more than 60 gene cassettes have been described [7]. Class 1 integrons prevail most in clinical Gram-negative bacteria isolates and consist of two conserved segments. The 5′ conserved region encodes a

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site-specific recombinase (integrase) and a strong promoter or promoters that ensure expression of the integrated cassettes. The 3′ conserved segment contains the quaternary ammonium compound resistance gene *qac EΔ1*, the sulfonamide resistance gene *sul-1* and an open reading frame of unknown function [2,3].

Currently, there is little information regarding the mechanism of acquisition and dissemination of antibiotic resistance in calf pathogenic *E. coli* strains in China. In this study, 22 pathogenic *E. coli* strains isolated from the Beijing surrounding region in China were investigated for associated disease serotype, virulence factors and antimicrobial susceptibility pattern. In addition, the presence of class 1 integron and content of antibiotic resistance cassettes were characterized to determine whether the resistance phenotypes observed could be attributed to the acquisition of integron-mediated resistance gene cassettes.

2. Materials and methods

2.1. Bacterial strains

Twenty-two *E. coli* strains were isolated from calf diarrhea cases in the Beijing surrounding region in China and were identified by the China Institute of Veterinary Drug Control and China Agricultural University. The strains were stored at −86 °C until analysis.

2.2. Antimicrobial susceptibility determination and serotyping

Antimicrobial minimal inhibition concentrations (MICs) for *E. coli* strains were determined using the standard broth doubling dilution method on Muller–Hinton medium and interpreted according to the NCCLS standards [8]. *E. coli* ATCC 25922 was used as control in MICs determinations. The O-antigen serotyping and virulence factor analysis were performed with commercially available monovalent test sera following the manufacturer's operations and analytical guides.

2.3. Detection of the integrase gene and gene cassettes

The template DNA for PCR was prepared as described previously [9]. The general strategy for PCR detection of the class 1 integron was according to the method described by Levesque et al. [10]. Primers used for PCR detection are listed in Table 2. The PCR was carried out in a final volume of 50 μL containing 1 μg template DNA, 100 pmol of each primer, 1× PCR buffer, 0.2 mM of each dNTP (dATP, dCTP, dGTP, dTTP) and 2.5 U Ex Taq polymerase (Takara, Japan). For the amplification of the different amplicons, the appropriate program parameters were used accordingly. A total of 32 cycles were performed in the PCR Express (HYBRID Corporation).

The PCR products were electrophoresed on a 1.5% agarose gel. The DNA band of interest was excised, purified and ligated to pGEM-T Easy vector (Promega). JM109 competent cells were transformed, and the white clones were screened and digested with *Eco*RI for identification. All the amplicons positive with the specific primers were sequenced.

2.4. DNA Sequencing and data analysis

Nucleotide sequences were sequenced with the ABI PRISM Big Dye Primer Cycle Sequencing Ready Reaction Kit and ABI 377 DNA auto-sequencing machine using T7 and SP6 sequence primers (Perkin Elmer company). For analysis of the data, the software DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715, USA) and the program NCBI-BLAST (www.ncbi.nlm.nih.gov) were used.

3. Results

3.1. Antimicrobial susceptibility determination and serotyping

Characteristics of the *E. coli* isolates positive for int I1 ($n = 13/22$) are shown in Table 1. These *E. coli* isolates were multi-resistant and exhibited resistance to four to seven commonly used antimicrobial agents. Serotype identification revealed O101 as the predominant serotype ($n = 6/13$). Most isolates carried the adhesin antigen K99 ($n = 9/13$) and F41 ($n = 7/13$).

3.2. Detection and characterization of the integrase gene and gene cassettes

As shown in Table 1, 59% ($n = 13/22$) of the isolates were positive for the presence of *int I1* and *qacEΔ1–sul-1*, which are markers for class 1 integrons. Consequently, the integron-borne gene cassettes were cloned and sequenced. The integrons were found to contain two to three gene cassettes and the combinations of these gene cassettes are shown in Table 1. Four distinct kinds of gene cassette arrays were characterized. These were aadB–aadA1–cmlA, aadB–ORF–cmlA, dfrA1–ORF and dfrA17–aadA5. Of these, dfrA1–orf (45%) and aadB–orf–cmlA (32%) were found most prevalent. With the exception of two open reading frames of unknown function, these gene cassettes encoded trimethoprim resistance (*dfrA1*, *dfrA17*), spectinomycin/streptmycin resistance (*aadA1*, *aadA5*), kanamycin /gentamicin/tobramycin resistance (*aadB*) and non-enzymic chloramphenicol resistance (*cmlA*).

Table 1
Characteristics of the *E. coli* isolates positive for int I1 in this study

Isolates	Source	Serotype	MICs ($\mu g\,ml^{-1}$) of :						int I1	Size of CS-PCR product (bp)	Inserted gene cassettes and order	*qacEΔ1–sul1*
			Ap	Su	Tc	St	Gm	Cm	Ff			
C260	Beijing	O101:k99, F41	>32	>512	>32	>256	>32	>128	128	+	3.2k, 1.3k	aadB–aadA1–cmlA,dfrA1-orf	+
C261	Beijing	O101:k99, F41	>32	>512	>32	>256	32	128	2	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+
C262	Beijing	O101:k99, F41	>32	>512	>32	>256	8	128	32	+	1.8k, 1.3k	dfrA17–aadA5, dfrA1–orf	+
C286	Beijing	O38:k99, F41	4	>512	>32	>256	>32	32	32	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+
C287	Beijing	O101:F41	>32	>512	>32	>256	>32	128	2	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+
C288	Beijing	O38:K99, F41	>32	>512	>32	>256	8	2	2	+	1.8k	dfrA17–aadA5	+
C207	Hebei province	O15:K99	>32	>512	>32	>256	>32	>128	64	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+
C209	Hebei province	O101:K99	>32	>512	>32	>256	>32	>128	>128	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+
C306	Hebei province	O44:F17	>32	>512	>32	128	4	2	2	+	1.3k	dfrA1–orf	+
C307	Hebei province	O87:F17	>32	>512	>32	>256	8	128	4	+	1.8k, 1.3k	dfrA17–aadA5, dfrA1–orf	+
C312	Hebei province	O128:K99, F41	>32	>512	>32	128	4	4	2	+	1.3k	dfrA1–orf	+
C914	Hebei province	O101:K30, K99	>32	>512	>32	>256	>32	>128	128	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+
C916	Hebei province	O20:K101, 987P	>32	>512	>32	>256	>32	16	64	+	3.0k, 1.3k	aadB–orf–cmlA, dfrA1–orf	+

[a] Abbreviation for antibiotics: Ap, ampicillin; Su, sulfamethoxazole; Tc, tetracycline; St, streptomycin; Gm, gentamycin; Cm, chloramphenicol; Ff, florfenicol.

A search for homologous sequences in GenBank revealed: (1) the gene cassette array aadB–ORF–cmlA presented 99% identity with that found in *Salmonella* sp. isolates from America (AJ487033); (2) the gene cassette array dfrA1–ORF presented 99% identity with those found in *E. coli* isolates from Japan (AB161449) and China (AY602403) or *Salmonella enterica* subsp. enterica serovar isolates from France (AY146989) or *Vibrio cholerae* isolates from India (AF455254) and Thailand (AF221901); (3) the gene cassette array dfrA17–aadA5 presented 100% identity with those found in *E. coli* isolates from Austria (AF169041) and Taiwan (AF170088) or *Salmonella* sp. isolates from China (AY263739) and Japan (AB127354) or *Klebsiella pneumoniae* isolates from Korea (AF180469) and France (AF220757); (4) the gene cassette array aadB–aadA1–cmlA has not been reported previously to our knowledge.

4. Discussion

At present, the class 1 integrons may potentially have the capability to integrate nearly all the antimicrobial resistant genes such as β-lactams, aminoglycosides, phenicols, sulfonamides, macrolides, trimethoprim and rifampin [6,10–13] to disseminate multidrug resistance in clinical bacteria isolates.

The investigation in this study revealed that aminoglycoside resistance determinants (*aadB*, *aadA1* and *aadA5*) and trimethoprim resistance determinants (*dfrA1*, *dfrA17*) and non-enzymic chloramphenicol resistance determinants (*cmlA*) were prevalent among calf pathogenic *E. coli* strains. This may be due to the fact that aminoglycosides, chloramphenicol and trimethoprim were often widely used for the prevention and treatment of diarrhea in calf in past years.

However, the class 1 integrons examined in this study did not account for the total resistance phenotype observed among the clinical calf pathogenic *E. coli* isolates, and this could possibly reflect the presence of other mobile DNA elements or resistance genes that we could not detect [9].

A search for published homologous sequences in GenBank revealed that the identical gene cassette arrays were also found in the same bacterial species with different serotype, in different bacterial species (*E. coli*, *Salmonella* sp., *K. pneumoniae* or *V. cholerae*), in different hosts (animals or humans) or in different geographic areas. These data seemed to suggest that class 1 integrons may play an important role in contributing to the horizontal dissemination of antibiotic resistance in the same bacterial species with different serotype, different bacterial species, different hosts or different geographic areas via international travel or trade [14,15].

Table 2
PCR Primers used for amplification of class 1 integrons

Gene	PCR Primers[a]	Expected size of PCR product (bp)	Reference
5′ CS	TCATGGCTTGTTATGACTGT		[16]
3′ CS	GTAGGGCTTATTATGCACGC		
Int I1	F: CCTCCCGCACGATGATC	280	[9]
	R: TCCACGCATCGTCAGGC		
qacEΔ1–sul1	F: CTCACAGCCAAACTATCAGGTC	1269	This study
	R: GAATGCTAGGCATGATCTAACC		

[a] F, forward; R, reverse.

In conclusion, the study demonstrated the wide distribution of the class 1 integrons among calf pathogenic *E. coli* isolates from the Beijing surrounding region in China. The genetic content and combination and emergence frequency of these gene cassettes may reflect antibiotic selective pressure in this specific region, providing useful antimicrobial surveillance information for the rational and effective use of antimicrobials.

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