Detection of Integrons from *Escherichia coli* Isolates Obtained from Humans and Animals in the Republic of Korea

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Abstract

Integrons were detected in 153 of 1134 *Escherichia coli* isolates obtained from feces of humans and animals. Healthy humans were 50% integron positive while 36% of isolates from patients were integron positive. Among the isolates obtained from cows, more number of integrons were detected in beef cattle.

Keyword: Integron

1. Introduction

In human and veterinary medicine the wide application of antibiotics has led to large-scale dissemination of bacteria resistant to antibiotics in the environment. It was reported that antibiotic resistant strains reach the environment through manure and liquid manure of animals as well as through human excretions and even those bacteria can also be found in the feces of healthy persons and the intestinal flora of healthy individuals [1]. Ploy et al. (2000) found that dissemination of antibiotic resistance genes by horizontal transfer led to the rapid emergence of antibiotic resistance among clinical isolates of bacteria [2]. Feuerpfeil and Stelzer (1992) found that 80.5% of feces samples of healthy persons contained bacteria resistant to antibiotics of which 98% were *E. coli* [3]. In *E. coli*, there is higher possibility of the antibiotic resistant genes to be spread. It was studied that the spread of resistance genes is greatly enhanced when they form part of a mobile gene cassette, since this provides for horizontal transfer by several mechanisms which include (i) mobilization of individual cassettes by the integron-encoded integrase [4], (ii) movement when the integron containing the cassette relocates—probably by targeted transposition [5, 6, 7], (iii) dissemination of larger transposons such as Tn*21* carrying integrons [8], and (iv) movement of conjugative plasmids containing integrons among different bacterial species. It is therefore not surprising that many of the antibiotic resistance genes found in clinical isolates of gram-negative microorganisms are part of a gene cassette inserted into an integron [9]. The four classes of integron so far identified (classes 1, 2, 3, and 4) are distinguished by their respective integrase (*int*) genes [10, 11, 4]. Class 4 is a

distinctive class of integrons located in the *Vibrio cholerae* genome and is not known to be associated with antibiotic resistance [11]. Despite the detailed understanding of the molecular relationship between gene cassettes and integrons [12, 9], there is a paucity of information about diversity and distribution of integrons in hosts. Only a few studies have suggested that integrons are widespread in both animal and human clinical bacterial isolates [13, 14, 15, 16]. The purpose of this current study was to determine the incidence of integrons from *E. coli* isolates obtained from humans and animals in the Republic of Korea.

2. Experimental procedures

Samples were collected from healthy humans, patients, dairy cattle and beef cattle. Collected samples were preserved in ice and streaked within 12 h of collection onto mFC agar and were incubated overnight at 44.5°C and then the blue colonies were streaked onto the surfaces of MacConkey agar plates and transferred onto ChromAgar ECC. *E. coli* isolates form blue colonies on ChromAgar ECC, which differentiates them from other coliform and gram-negative bacteria, which form red and colorless colonies, respectively. After overnight incubation at 37°C, pink colonies that were obtained from the MacConkey plates and were also positive for *E. coli* on ChromAgar ECC plates, which were used to inoculate citrate agar, EC broth supplemented with 4-methylumbelliferyl-D-glucuronide and 1% tryptone, and methyl red—Voges-Proskauer broth. Isolates that did not grow on citrate agar, were positive for gas production and fluorescence on EC broth containing 4-methylumbelliferyl-D-glucuronide, produced indole from tryptophan, and produced acidic end products when they were grown in methyl red–Voges-Proskauer broth. They were designated E. coli isolates and used for subsequent studies.

DNA was extracted from *E. coli* isolates obtained from the samples by boiling whole cells in 0.05 N NaOH for 15 minutes at 95°C. 10-fold diluted DNA solutions in TE buffer was used as a template for PCR integron detection. PCR was done with the degenerate primers hep35 (5' TGCGGGTYAARGATBTKGAT TT 3') and hep36 (5' CARCACATGCGTR TARAT 3'), which hybridize to conserved regions of integron-encoded integrase genes *intI1*, *intI2*, and *intI3* [17]. Integrons were observed by analyzing integrase PCR products by gel electrophoresis (Figure 1).

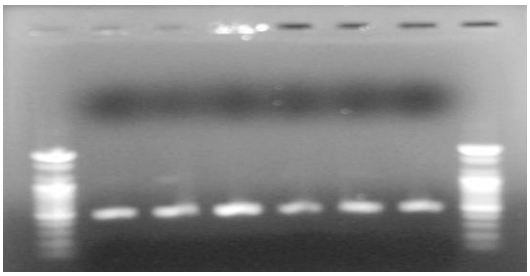

Figure 1 Gel electrophoresis of integrase PCR products using 10 bp DNA ladder

3. Results and Discussions

A total of 1134 fecal isolates were obtained from humans and animals in the Republic of Korea (Table 1). In this present study it was observed that 13.49% of these isolates were integron positive. In contrast, Ebner et al. (2004) detected 32.8% isolates of integrons from different animals [18]. We observed integrons in 100 isolates of 596 isolates obtained from cows.

Table 1 Integron positive isolates from humans and animals

Sources	No. of isolates	Integrons
Human	538	53
Dairy cattle	284	30
Beef cattle	312	70

Čížek et al. (2007) observed a high prevalence of resistant coliform bacteria in milk filters obtained on Czech dairy farms [19]. In our study it was observed that isolates of beef cattle were more vulnerable to be integron positive than those collected from feces of dairy cattle.

Of the 538 isolates from humans, 83 isolates were from patients. We detected integrons in 30 of 83 isolates (36.14%) of the patients. On the other hand, only 23 isolates from 455 isolates (5.05%) of healthy humans were integron positive (Figure 2). In comparison White et al. (2000) detected integrons in 59 of 120 urinary isolates (49%) from *Entero-bacteriaceae* collected from patients in hospitals [17] from which it can be assumed that patients are more vulnerable to be integron positive.

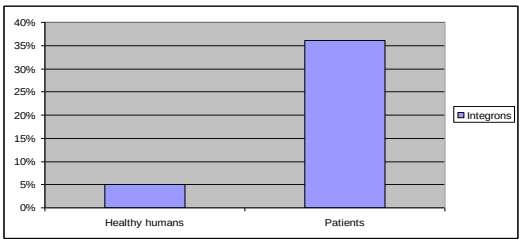

Figure 2 Percentage of integron positive isolates in healthy humans and patients

The dissemination of antibiotic resistance genes among bacterial strains is an increasing problem in infectious diseases. Many antibiotic resistance genes are located on plasmids and on transposons, enabling their transfer among a variety of bacterial species. Gene cassettes can exist as free circular molecules and are transcribed only when captured and inserted into an integron [4]. New cassettes are continually being discovered. Nowadays over 60 cassettes have been discovered [9]. Integrons are natural expression vectors that permit the insertion of antibiotic resistance genes by a site-specific recombinational mechanism. With the potential of integrons to capture and collect gene cassettes there is possibility that antibiotic resistant, genes can be widespread in nature. *E. coli*, which are deadly pathogens if antibiotic resistant can be very dangerous for the environment. Antimicrobial resistance genes allow a microorganism to expand its ecological niche, allowing its proliferation in the presence of certain noxious compounds. The problem can be further increased with the widespread existence of integrons since antibiotic resistant genes can be propagated by integrons.

Since we detected integrons in lots of isolates of humans and animals in the Republic of Korea, our future work will be directed to the identification of the transposon structures that contain these integrons in *E. coli* and other bacterial isolates.

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