Detection of Integrons from *Escherichia coli* Isolates Obtained from Some Selected Animals in Republic of Korea

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Abstract We detected integrons in 298 of 1106 *Escherichia coli* isolates obtained from the feces of pigs, chicken, ducks and elks. Among the sources there was higher number of integrons detected in the isolates of pigs. No integron was found in the isolates of geese. Detection of lot of integrons in these isolates discovers the possibility of spread of antibiotic resistance genes in the environment.

Keyword Integron

Introduction

Antibiotic resistant strains reach the environment through manure and liquid manure of animals as well as through human excretions and even these can also be found in the feces of healthy persons and the intestinal flora of healthy individuals [1]. It was observed 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) observed that 80.5% samples of feces of healthy persons

contained bacteria resistant to antibiotics of which 98% were *E. coli* [3]. It was found that the spread of resistance genes is greatly enhanced when they form part of a mobile gene cassette. It is 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 [4]. Gene cassettes can exist as free circular molecules and are transcribed only when captured and inserted into an integron [5]. New cassettes are continually being discovered. Nowadays, over 60 cassettes have been discovered [4]. Integrons are natural expression vectors that permit the insertion of antibiotic resistance genes by a site-specific recombinational mechanism. Only a few studies suggested that integrons are widespread in both animal and human clinical bacterial isolates [6–9]. The purpose of this current study was to determine the incidence of integrons from *E. coli* isolates obtained from some selected animals in Republic of Korea.

Materials and Methods

Sample Collection

Samples of feces were collected from pigs, chicken, ducks, elks and geese in South Korea.

Isolation of *E. coli*

Samples were stored on ice and streaked within 12 h of collection onto mFC agar. After overnight incubation at 44.5°C, 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

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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 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 were designated *E. coli* isolates and used for subsequent studies.

Extraction of DNA

DNA was extracted from *E. coli* isolates obtained from the samples by boiling whole cells in 0.05 N NaOH for 15 min at 95°C.

Restriction Fragment Length Polymorphism

Tenfold diluted DNA solutions in TE buffer was used as a template for PCR integron detection. PCR was done with the degenerate primers hep35 (59 TGCGGGTYAARGAT BTKGATTT 39) and hep36 (59 CARCATGCGTRT ARAT 39), which hybridize to conserved regions of integron-encoded integrase genes *int11*, *int12*, and *int13* [10]. Integrons were observed by analyzing integrase PCR products by gel electrophoresis.

Visualization

Photographs were taken under the ultraviolet lighting conditions after electrophoresis. For integron positive isolates we targeted 491 base pairs.

Results and Discussion

1106 fecal isolates were obtained from pigs, chicken, ducks, geese and elks. We detected integrons in 298 of 1106 isolates. In this present study it was observed that 26.94% of these isolates were integron positive. Ebner et al. [11] detected 32.8% isolates containing integrons from various animals.

In our study integrons were observed higher in pigs than other animals (Fig. 1). 39.85% integron positive samples were observed in pigs. Chicken showed relatively low integron positive values. In chicken 12.14% isolates were integron positive. 23.14% *E. coli* isolates obtained from feces of ducks were integron positive. Integrons were

Fig. 1 Percentage of integrons isolated from animals

detected in 14.81% isolates in elks. No integron was found in the isolates from geese.

The number of integrons that we have detected can be highly threatening. These values show the higher probability of antibiotic resistance genes to be spread among animals. The acquisition and spread of antibiotic resistance genes among bacteria that are intimately associated with humans and their domesticated animals are well reported [12]. 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. Since we detected integrons in lot of isolates of animals in 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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References

1. Reinthaler FF, Posch J, Feierl G, Wust G, Haas D, Ruckebauer G, Mascher F, Marth E (2003) Antibiotic resistance in *E. coli* in sewage and sludge. *Water Res* 37:1685–1690
2. Ploy MC, Lambert T, Couty JP, Denis F (2000) Integrons: an antibiotic resistance gene capture and expression system. *Clin Chem Lab Med* 38:483–487
3. Feuerpfeil I, Stelzer W (1992) Presence of antibiotic-resistant coliform bacteria in the human intestinal flora (Das Vorkommen von antibiotikaresistenten koliformen Bak-terien in der Darmflora des Menschen). *Bundesgesund-heitsbl* 35:61–65
4. Recchia GD, Hall RM (1995) Gene cassettes—a new class of mobile element. *Microbiology* 141:3015–3027
5. Collis CM, Hall RM (1992) Gene cassettes from the insert region of integrons are excised as covalently closed circles. *Mol Microbiol* 6:2875–2885
6. Bass L, Liebert CA, Lee MD, Summers AO, White DG, Thayer SG, Maurer JJ (1999) Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrob Agents Chemother* 43:2925–2929

7. Levesque C, Piche L, Larose C, Roy PH (1995) PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 39:185–191
8. Martinez-Freijo P, Fluit AC, Schmitz FJ, Grek VSC, Verhoef J, Jones ME (1998) Class 1 integrons in Gram-negative isolates from different European hospitals and association with decreased susceptibility to multiple antibiotic compounds. *J Antimicrob Chemother* 42:689–696
9. Senda K, Arakawa Y, Ichiyama S, Nakashima Hito, Ohsuka S, Shimokata K, Kato N, Ohta M (1996) PCR detection of metallo-beta-lactamase gene (*blaIMP*) in gram-negative rods resistant to broad-spectrum beta-lactams. *J Clin Microbiol* 34:2909–2913
10. White PA, McIver CJ, Deng YM, Rawlinson WD (2000) Characterisation of two new gene cassettes, *aadA5* and *dfrA17*. *FEMS Microbiol Lett* 182:265–269
11. Ebner P, Garner K, Mathew A (2004) Class 1 integrons in various *Salmonella enterica* serovars isolated from animals and identification of genomic island SGI1 in *Salmonella enterica* var Meleagridis. *J Antimicrob Chem* 53:6
12. Čížek A, Dolejšká M, Novotná R, Haas D, Vyskočil M (2007) Survey of Shiga toxigenic *Escherichia coli* O157 and drug-resistant coliform bacteria from in-line milk filters on dairy farms in the Czech Republic. *J Appl Microbiol* 104(3):852–860