

Detection of β -lactam Antibiotic-resistant Genes in *Escherichia coli* using DNA Chip from Porcine Fecal Samples

Sung-ho Na, Ho-seong Cho, Yong-hwan Kim¹, A. W. E. Effendy and Nam-yong Park

Dept. of Vet. Pathol., College of Vet. Med., Chonnam National Univ., Gwangju, Korea

¹Dept. of Vet. Research, Gwangju City Institute of Health and Environment, Gwangju, Korea

E-mail: nypark@chonnam.ac.kr

Introduction

There prevalence of β -lactamases bacteria in animals has been increased since 1990s [1]. The resistance in *E. coli* which is mediated by β -lactamases hydrolyze the β -lactam ring eventually inactivate the antibiotics [2]. Generally, β -lactamases can be classified into four main groups and eight subgroups according to their functional and structural characteristics [3]. The detection of β -lactam antibiotic-resistant bacteria by DNA chip has been described [4]. The chip has a specific probe DNAs that contained the β -lactam antibiotic-resistant genes which was labeled by multiplex PCR reaction with a mixture of primer sets that were designed to amplify specific gene. Here we report the susceptibility of enteropathogenic *E. coli* isolated from pigs in Korea using the DNA chip in detecting β -lactam antibiotic-resistant genes.

Materials and Methods

40 samples of *E. coli* isolated and identified from diarrheic fecal samples of pigs were used in the study. All *E. coli* were subjected to antimicrobial sensitivity test based on MICs. The preparation of target DNA was according to Lee *et. al* [4]. The positive control DNA was also labeled with the same procedure as the target DNA, except 200 ng of the plasmid that contained the yeast DNA fragment S400. The 45 μ l target DNA and 1 μ l positive control were mixed and dried in SpeedVac, then suspended in a 20 μ l hybridization solution. The DNA chip used was developed by the BMS Korea Research Center, Korea. The antibiotic-resistant genes on the DNA chip were *PSE*, *OXA*, *FOX*, *MEN*, *CMY*, *TEM*, *SHV*, *OXY* and *AmpC* [4]. After hybridization and washing, the chip was scanned by the microarray reader ScanArray 5000 (Packard, USA) and analyzed with QuantArray software (Packard, USA).

Results

Thirty two out of 40 *E. coli*, DNA chip system detected the presence of at least one β -lactamases genes in the bacteria. Resistance to β -lactamases was mediated mainly by *AmpC* (100%), *TEM* (59%), *CMY* (34%), *PSE* (9%) and 6% for both *OXA* and *SHV* genes. 25% (8 of 40) from the isolates were found to have three or more resistant genes, while 56% (18 of 40) was detected two resistant genes. The bacteria that showed susceptibility during the antimicrobial disk testing did not represent by any targeted genes.

Discussion

β -lactams are widely used in human and veterinary medicine to treat human and animal infections [2]. Resistance to β -lactam antimicrobial agents in *E. coli* is primarily mediated by β -lactamase, which hydrolyze the β -lactam ring thus inactivate the antibiotic [2]. In this study, the highest expression of *AmpC* genes represent significant role of these isolates in the resistance phenomena to β -lactams. Organisms overexpressing *AmpC* β -lactamases are a major clinical concern because these organisms are usually resistant to all the β -lactam drugs, except for cefepime, ceftiofime, and the carbapenems [5]. The presence of more than one β -lactamases resistant genes in a single bacterium are also a great concern since most of the problems usually related to indiscriminate use of broad spectrum in livestock industry. In this study, the DNA chip system provides rapid and reliable method for veterinarian to determine the drug of choice when dealing with monitoring and surveillance of animal disease.

References

1. Bush, K, et al., J. Antimicrob Chemother. 1997, **39**, 13-18.
2. Ferial, G., et al., J. Antimicrob Chemother. 2002, **49**, 77-85.
3. Girlich, D., et al., Antimicrob Agents Chemo. 2000, **44**, 3220-3223.
4. Lee, Y., et al., Mol. Cells, 2002, **14**, 192-197.
5. Livermore, D. M. Clin Microbiol Rev. 1995, **8**, 557-584.