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Original Article

Predicting Phenotypic Antimicrobial Resistance in *Escherichia coli* Isolates, Using Whole Genome Sequencing Data

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전장유전체 분석을 통한 대장균의 항균제 내성 표현형의 예측

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ABSTRACT

Background: The application of genotypic antimicrobial sensitivity tests (ASTs) is dependent on the reliability of the predictions of phenotypic resistance. In this study, routine AST results and the presence of corresponding antimicrobial resistance genes were compared.

Methods: Eighty-four extended-spectrum-β-lactamase-producing *Escherichia coli* isolates from poultry-related samples were included in the study. The disk diffusion method was used to test for susceptibility to antimicrobial compounds, except colistin susceptibility, which was tested using the agar dilution method. Whole-genome sequencing (WGS) was performed using a NextSeq 550 instrument (Illumina, USA). Antimicrobial resistance genes were detected using ResFinder 4.1.

Results: Concordance rates between the genotype and phenotype ranged from 35.7% (ciprofloxacin) to 96.4% (tetracycline). The presence of *tet* was a good predictor of phenotypic resistance.

Conclusion: The genotype was a good predictor of tetracycline phenotypic resistance, but there was a gap in the prediction of phenotypic ASTs for trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin, and ciprofloxacin. We concluded that WGS-based genotypic ASTs are inadequate to replace routine phenotypic ASTs.

Keywords: Antimicrobial resistance, Phenotype, Genotype, Whole genome sequencing, *Escherichia coli*

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INTRODUCTION

Accurate antimicrobial susceptibility of pathogen is very important to apply effective antimicrobials to infected patients. Antimicrobial susceptibility testing (AST) is routinely based on the phenotypic method, which needs overnight incubation and tight control of experiments [1]. Recently, rapid genotypic AST has been proposed with introduction of various molecular methods [2].

Polymerase chain reaction (PCR)-based molecular AST assay usually detects well-selected specific antimicrobial resistance (AMR) genes and whole genome sequencing (WGS) detects a wide range of AMR genes, both of which can detect indirect surrogate markers for phenotypic AST [3]. However, genotypic AST might be influenced by the level of gene expression. There could be a discrepancy between genotype and phenotype AST. Functional ability is largely dependent on the AMR gene database in analytical programs [4]. Therefore, the reliability of predicting phenotypes should be evaluated before genotypic AST is applied.

In this study, we compared routine phenotypic AST with the presence of corresponding AMR genes or mutation detected by WGS. The purpose of this study was to provide an overview about the reliability of genotypic AST.

MATERIALS AND METHODS

A total of 84 extended-spectrum-β-lactamase producing *Escherichia coli* (ESBL-EC) were included in this study, isolated from poultry, poultry farm environment, or workers from January to August 2019 during the project collaborated with the Korea Disease Control and Prevention Agency [5]. Species identification was performed by a MALDI Biotyper (Bruker Daltonik, Bremen, Germany). The disk diffusion method was used for antimicrobial susceptibility of cefotaxime, ceftazidime, cefepime, cefoxitin, aztreonam, imipenem, meropenem, ertapenem, amikacin, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, tetracycline, tigecycline, chloramphenicol, and nitrofurantoin. The diameter of inhibition zone was interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria [6]. To detect colistin resistant isolates, test organisms were screened on Mueller-Hinton agar (Oxoid, Basingstoke, UK) containing colistin (0, 1, 2, and 4 μg/mL) using *E. coli* ATCC25922 strain as an internal control. If minimal inhibition concentration was > 2 μg/mL, the isolate was regarded as colistin-resistant organism according to CLSI breakpoints for *Pseudomonas aeruginosa* and *Acinetobacter* spp. because there were no CLSI breakpoints for *Enterobacteriaceae* [6].

ESBL production was confirmed by PCR and sequencing of ESBL genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) for any isolate showing resistance to cefotaxime or ceftazidime as described in a previous study [7]. For WGS, DNAs of freshly sub-cultured isolates were extracted using a GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA) and 8 μg of input genomic DNA was used. Entire genomes of ESBL-EC isolates were sequenced using a NextSeq 550 instrument (Illumina, San Diego, CA, USA). Sequences were assembled with Spades (version 3.11.1) and annotated with Prokka (version 1.13.7). Data of antimicrobial resistance genes were obtained from the website of Center for Genomic Epidemiology [8], including ResFinder 4.1 with 90% ID threshold and 60% minimal length [9].

In this analysis, carbapenem, amikacin, tigecycline, nitrofurantoin, and colistin were excluded because nearly all isolates were susceptible to these antimicrobials except that one isolate showed resistance to nitrofurantoin. The presence of resistance genes was compared with results of phenotypic antimicrobial susceptibility test. When a related resistance gene or mutation was present in a phenotypic resistant isolate, the isolate was defined as a concordant isolate. When multiple resistance genes were involved in phenotypic resistance, any of resistance genes was regarded as a possible gene.

This study was approved by the Institutional Review Board of National Health Insurance Service Ilsan Hospital, Goyang, Korea as required by hospital policy (IRB No. NHIMC 2022-07-018).

RESULTS

Resistance genes for corresponding antimicrobial phenotypes were detected, including β-lactam (*bla*$_{OXA-1}$, *bla*$_{TEM-1}$, *bla*$_{CMY-2}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-27}$, *bla*$_{CTX-M-55}$, and *bla*$_{CTX-M-65}$), aminoglycoside (*aadA1, aadA2, aadA5, aadA12, aac(6')Ib-cr, aac(3')-IIa, aac(3')-IId, aac(3')-IIe, aac(3')-IVa, aph(3')-Ia , aph(4)-Ia*), quinolone (*aac(6')Ib-cr, qnrB19, qnrS1, qnrS2*), trimethoprim-sulfomethoxazole (*dfrA*1, *dfrA*12, *dfrA*14, *dfrA*17, *sul1, sul2*, *sul3*), tetracycline (*tet(A), tet(B)*), and chloramphenicol (*catA1, catB3*). Chromosomal mutations of *parC*, *parE*, gyr*A*, and *gyrB* for ciprofloxacin resistance were also searched.

Concordance rates ranged from 35.7% (ciprofloxacin) to 52.4% (trimethoprim-sulfamethoxazole), 51.2% (chloramphenicol and gentamicin), and 96.4% (tetracycline) when any related resistance gene was considered to be responsible for the resistance phenotype.

The effect of individual resistance gene was summarized as concordance rate (Table 1). The presence of *tet*, *sul* plus *dfr*, *cat, qnrB19*, and *qnrS2* well predicted phenotypic resistance.

Table 1. Concordance rates between phenotypic antimicrobial susceptibility and genotype with the presence of resistance genes or mutations

Concordance rates: % (n/n)*				
GM-R	CP-R	SXT-R	TET-R	CIP-R
aac(3')-IIa: 100 (1/1)	*catA1*: 100% (2/2)	*sul* only: 3 (1/35)	*tet(A)*: 99 (82/83)	*parC*: 50 (1/2)
aac(3)-IId: 100 (2/2)	*catB3*: 100% (3/3)	*sul2*: 0 (0/32)	*tet(A)+tet(B)*: 100 (1/1)	*qnrB19*: 100 (2/2)
aac(3)-IId+aadA2: 67 (2/3)		*sul3*: 50 (1/2)		*qnrS1*: 0 (0/3)
aac(3)-IId+aadA5: 100 (1/1)		*sul1+sul2*: 0 (0/1)		*qnrS2*: 100 (1/1)
aac(3)-IIe: 50 (1/2)		*dfr* only: 33 (1/3)		*qnrS2+aac(6')Ib-cr*: 100 (3/3)
aac(3)-Iva+aadA1+aadA2 +aph(4)-Ia: 100 (1/1)		*dfrA12*: 50 (1/2)		*aac(6')Ib-cr*: 0 (0/1)
		dfrA14: 0 (0/1)		
aac(3)-Iva+aadA2+aph(4)-Ia; 33 (1/3)		*sul+dfr*: 88 (29/33)		
aac(3)-Iva+aph(4)-Iva: 27 (4/15)		*sul1+dfrA17*: 100 (1/1)		
aac(3)-VIa+aadA1:100 (1/1)		*sul2+dfrA1*: 100 (1/1)		
aac(6')Ib-cr: 0 (0/1)		*sul2+dfrA12*: 100 (1/1)		
aac(6')Ib-cr+aadA5: 0 (0/3)		*sul2+dfrA14*: 100 (5/5)		
aadA1: 0 (0/3)		*sul2+dfrA17*: 100 (5/5)		
aadA1+aadA5: 0 (0/1)		*sul3+dfrA17*: 100 (1/1)		
aadA2: 14 (1/7)		*sul1+sul2+dfrA12*: 75 (9/12)		
aadA5: 0 (0/7)		*sul1+sul2+dfrA14+drfA17*: 50 (1/2)		
aadA5+aph(3')-Ia: 0 (0/1)		*sul1+sul2+dfrA17*: 100 (5/5)		
aadA12: 0 (0/2)				
aph(3')-Ia: 0 (0/1)				

*The number of isolates with phenotypical resistance/the number of isolates with resistance genes or mutations.
Abbreviations: GM-R, phenotypic resistance to gentamicin; CP-R, phenotypic resistance to chrolamphenicol; SXT-R, phenotypic resistance to trimethoprim-sulfamethoxazole; TET-R, phenotypic resistance to tetracycline; CIP-R, phenotypic resistance to ciprofloxacin.

All ESBL-EC isolates were resistant to cefotaxime but susceptible to cefoxitin. However, susceptibilities to ceftazidime, cefepime, and aztreonam were different according to CTX-M type. CTX-M-55 producers showed high resistance rates to these three lactams (Table 2).

Table 2. Concordance rates between resistance phenotype to cephalosporins and genotypes of CTX-M and PACBL

CTX-M type	CTX-R % (n)	CAZ-R % (n)	CEF-R % (n)	FOX-R % (n)	AZT-R % (n)
CTX-M-1 (n=11)	100 (11)	0	18 (2)	0	18 (2)
CTX-M-14 (n=29)	100 (29)	0	4 (1)	0	0
CTX-M-15 (n=8)	100 (8)	25 (2)	88 (7)	0	100 (8)
CTX-M-27 (n=3)	100 (3)	0	67 (2)	0	67 (2)
CTX-M-55 (n=24)	100 (24)	42 (10)	75 (18)	0	79 (19)
CMY-2 plus CTX-M-55 (n=4)	100 (4)	100 (4)	0	100 (4)	100 (4)
CTX-M-65 (n=5)	100 (5)	0	0	0	20 (1)

Abbreviations: PACBL, plasmid-mediated AmpC-like β-lactamase; CTX-R, phenotypic resistance to cefotaxime; CAZ-R, phenotypic resistance to cefotazidime; CEF-R, phenotypic resistance to cefepime; FOX-R, phenotypic resistance to cefoxitin; AZT-R, phenotypic resistance to aztreonam.

DISCUSSION

To choose effective antimicrobials for patients with bacterial infection and survey resistant organisms, AST is very important [1]. Genotypic AST can be used to detect corresponding genes for antimicrobial resistance. It is applicable in a rapid manner without overnight incubation, which is usually needed in a phenotypic AST.

In the present study, the concordance between genotype and phenotype was not very good except for resistance to tetracycline. Low concordance rates were noted for resistance to trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin, and ciprofloxacin, which seemed to be mostly due to small number of cases. Others might be following reasons. First, detected multidrug efflux pump genes such as *floR*, *oqxA*, and *oqxB* were not included in comparison because their substrate specificity was not fully understood yet [10,11]. Second, the existence of genes was not equal to activity because gene expression levels might be different. Finally, there are many unknown resistance mechanisms and resistance genes. In this study, the possibility of unknown resistance genes was high for chloramphenicol-resistant or ciprofloxacin-resistant isolates because verified resistance genes were not detected in many of these isolates.

Bortolaia et al.[12] have reported that the concordance from 1,520 observations including 16 antimicrobials is 97%, ranging from 71.6% for cefepime and 100% for most antimicrobials in *E. coli*. Tyson et al.[13] have reported a specificity of 97.8% and a sensitivity of 99.6% for over 30 resistance genes and a number of resistance mutations in *E. coli*. Recently, Golden et al.[14] have reported high categorical agreements for ciprofloxacin, gentamicin, ceftriaxone, and trimethoprim/sulfamethoxazole in the evaluation of a total of 671 *E. coli* isolates. This study used a different definition, in which genotype and phenotype were determined to agree when an isolate was phenotypically non-susceptible and possessed known resistance genes or mutations, or when the same resistance genes or mutations were absent in a phenotypically susceptible isolate [14].

Our result showed that the presence of *tet*, *sul* plus *dfr*, *cat*, *qnrB19*, and *qnrS2* seemed to well predict phenotypic resistance, although the number of cases was limited. For aminoglycoside modifying enzymes, it was hard to find a tendency due to a small number of type-specific isolates. In this study, phenotypically resistance rather than non-susceptibility was used, which could contribute to the difference of the results in comparison of previous studies. The definition was used to evaluate the clear correlation between antimicrobial resistance genes and resistance phenotype to simply rule out the treatment options. *E. coli* is a major etiologic agents of urinary tract infection and effective antimicrobial therapy is possible with the 'intermediate' in susceptibility test [15].

The concordance rates between genotypes of CTX-M and cefotaxime resistance was very high as well-known [7], ceftazidime resistance varied according to the CTX-M types. CTX-M-55-producing isolates showed higher ceftazidime resistance rates than other types (Table 2).

In this study, genotype well predicted tetracycline phenotypic resistance, but there was a gap in prediction of phenotypic AST of trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin, and ciprofloxacin. We concluded that WGS-based genotypic AST is inadequate in replacement of routine phenotypic AST. Further study is needed to determine the effectiveness of WGS in identifying resistance genotypes of multidrug-resistant *E. coli* and whether these genotypes correlate with observed phenotypes.

요약

배경: 유전형 항균제 감수성을 적용하기 위해서는 이를 통해 표현형 내성을 얼마나 신뢰성 있게 예측할 수 있는지가 중요하다. 이 연구에서는 통상적인 항균제 내성 결과를 해당 항균제 내성 유전자 유무와 비교하여 보았다.

방법: 닭 관련 샘플에서 분리된 총 84주의 extended-spectrum-β-lactamase을 생성하는 *Escherichia coli*를 연구 대상으로 하였다. 표현형 항균제 감수성 시험은 디스크 확산법으로 하였고, colistin은 한천 희석법을 이용하였다. NextSeq 550 instrument (Illumina, USA)를 이용하여 전장유전체 분석을 시행하여 항균제 내성유전자는 ResFinder 4.1을 이용하여 검출하였다.

결과: 표현형과 유전형의 일치율은 35.7% (ciprofloxacin)에서 96.4% (tetracycline)로 다양하였고, *tet* 유전자의 존재가 가장 잘 표현형을 예측하였다.

결론: Tetracycline 감수성은 유전형으로 잘 예측되었으나, trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin, 및 ciprofloxacin에는 큰 차이를 보였다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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