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Correlation of Overexpression of Efflux Pump Genes with Antibiotic Resistance in *Escherichia coli* Strains Clinically Isolated from Urinary Tract Infection Patients[▽]

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***Escherichia coli* is one of the most common pathogens in urinary tract infections (UTIs), and antibiotic resistance in *E. coli* is becoming a serious problem in treating UTI. Efflux system overexpression is reported to contribute to *E. coli* resistance to several antibiotics. This study investigated the correlation of antibiotic susceptibilities with the overexpression of the efflux pump genes such as *marA*, *yhiU*, *yhiV*, and *mdfA* and with risk factors for antibiotic resistance in *E. coli* isolated from UTI patients. We examined the expression level of efflux pump genes using quantitative real-time reverse transcription-PCR (qRT-PCR). We also tested the *in vitro* susceptibilities to 12 kinds of antibiotics in 64 clinical strains of *E. coli* isolated from UTI patients. By multivariate analyses we revealed significant relationships between the overexpression of (i) *marA* and MICs of cefepime (FEP) and nalidixic acid (NAL), (ii) *yhiV* and MICs of minocycline (MIN), and (iii) *mdfA* and MICs of sitafloxacin (STX). In our investigation of the efflux pump genes, risk factors such as gender and the previous use of fluoroquinolones correlated with the overexpression of *marA*, and indwelling catheter use correlated with the overexpression of *mdfA*. In conclusion, we demonstrated that the increased expression of efflux pump genes such as *marA* and *mdfA* can lead to fluoroquinolone resistance in *E. coli*. These results contribute to our knowledge of the efflux system and raise the possibility of developing new agents, such as efflux pump inhibitors (EPIs), to antibiotic-resistant *E. coli*.**

There are many examples of multidrug resistance (MDR) in clinical isolates of *Enterobacteriaceae*, such as extended-spectrum β-lactamase (ESBL)-producing strains (6). The overexpression of efflux pumps was reported to contribute to MDR in *E. coli* (3, 12). Almost all Gram-negative bacteria have genes for efflux pumps belonging to the resistance nodulation division (RND) family (3), and seven homologous RND-type pumps are known in *E. coli*. Five of these (AcrB, AcrF, MdtB, MdtC, and YhiV) are known to expel many kinds of drugs. Most of these efflux genes are not expressed or are expressed at low levels in clinical isolates (3). The tripartite efflux pump, AcrAB-TolC complex, contains AcrA, a fusion protein; AcrB, a cytoplasmic membrane transporter protein; and TolC, an outer membrane channel (12). The overexpression of this pump often is associated with the MDR phenotype in *E. coli* (3, 15, 27, 32). YhiUV is regulated by the EvgAS, the two-component system (20), and helps expel doxorubicin and erythromycin by cooperating with TolC (3, 7, 10, 14, 20, 21). A previous report showed that a point mutation in *yhiV*, coding for YhiV, was associated with differences in the MDR phenotype (3). The overexpression of efflux pump genes such as *marA*, *yhiU*, *yhiV*, and *mdfA* is related to antibiotic resistance, especially fluoroquinolones (3, 12). This study investigated the relationship between the overexpression of these efflux pump genes and antibiotic resistance and risk factors for urinary tract infection (UTI) patients.

The characterization of efflux pumps could lead to the development of new weapons against antibiotic resistance, namely, efflux pump inhibitors (EPIs) (11, 24). EPIs efficiently inhibit the major AcrAB-TolC pump responsible for MDR in *E. coli* (24) and reduce resistance to antibiotics in bacterial isolates (6, 11).

In this study, we investigated the correlation of antibiotic susceptibilities with the expression of the efflux pump genes in *E. coli* of UTI patients by quantitative real-time reverse transcription-PCR (qRT-PCR) and with risk factors for antibiotic resistance.

MATERIALS AND METHODS

Bacterial isolates. Urine cultures were obtained from patients with urinary tract infection (UTI) treated at five hospitals in Hyogo prefecture in Japan from April to July 2008. Posttreatment isolates and other repeat isolates from the same patients were excluded from this study. Previous reports demonstrated that efflux pumps such as AcrAB-TolC were overexpressed in multidrug-resistant strains and were related to fluoroquinolone resistance (9, 22), and that the spread of fluoroquinolone resistance in *E. coli* strains isolated from UTI patients was greater in complicated UTI cases than in uncomplicated UTI patients (18, 29). Therefore, we selected strains mainly from complicated UTI cases to search for the overexpression of efflux pump genes, and we mainly selected LVX-resistant isolates from complicated UTI patients with the overexpression of efflux pump genes to compare the expression level of efflux pump genes in *E. coli* isolates to those of different clinical backgrounds. Twenty-nine LVX-resistant

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TABLE 1. Details of underlying urinary tract disease

Urinary tract underlying disease or condition	No. (%) of patients
Neurogenic bladder	26 (57.8)
Benign prostate hyperplasia	4 (8.9)
Hydronephrosis	4 (8.9)
Overactive bladder	3 (6.7)
Renal stone	2 (4.4)
Ureteral stone	2 (4.4)
Bladder stone	2 (4.4)
Bladder diverticulum	2 (4.4)
Vesicoureteral reflux	2 (4.4)
Ureteral stenosis	2 (4.4)
Chronic prostatitis	2 (4.4)
Prostate cancer	2 (4.4)
Atrophic bladder	1 (2.2)
Ureteral cancer	1 (2.2)
Pyonephrosis	1 (2.2)
Postrenal transplantation	1 (2.2)

and 16 LVX-susceptible isolates in complicated UTI and 9 LVX-resistant and 10 LVX-susceptible isolates in uncomplicated UTI were chosen from 156 isolates from UTI patients tested for this study. There were 45 patients with underlying urinary tract disease (complicated UTI). The details of underlying urinary tract disease in our study are shown in Table 1.

Susceptibility testing. Susceptibility testing was performed for 12 antibiotics, piperacillin (PIP), cefazolin (CFZ), ceftazidime (CAZ), cefepime (FEP), amikacin (AMK), gentamicin (GEN), imipenem (IPM), minocycline (MIN), nalidixic acid (NAL), ciprofloxacin (CIP), levofloxacin (LVX), and sitafloxacin (STX), according to Clinical and Laboratory Standards Institute (CLSI) guideline M7-A8, using frozen plates (Eiken Chemical Co. Ltd., Tokyo, Japan). The bacterial isolates were cultured on heart infusion agar plates at 37°C for 22 h, prepared as 1 ml of 1.0 McFarland standard, and then diluted in 9 ml of sterile saline and inoculated into frozen plates for 20 h. MICs of each antibiotic were analyzed by an IA 01 MIK mk II plate reader (Eiken Chemical Co. Ltd., Tokyo, Japan). *E. coli* ATCC 25922 was used as a quality control.

RNA extraction and quantification of RNA expression using qRT-PCR. The bacterial isolates were cultured at 37°C for 14 h on MacConkey agar plates, and a single colony on this plate was cultured in 4 ml of Luria-Bertani medium for 8 h. Total RNA was obtained from 1 ml (about 10^8 cells/ml) of mid-logarithmic-growth-phase bacterial cultures with RNA protect bacteria reagent and an RNeasy mini kit (Qiagen, Tokyo, Japan) (3, 12).

The expression level of RNA coding for the *marA*, *yhiU*, *yhiV*, and *mdfA* genes was estimated by qRT-PCR using a QuantiTect SYBR green reverse transcription-PCR kit (Qiagen, Tokyo Japan) in a 7500 real-time PCR system (Perkin-Elmer, Applied Biosystems, Warrington, United Kingdom). The RT-PCR mixture (25 μl) contained 1 μl of both forward and reverse gene-specific primers, 1 μl of QuantiTect RT mix, 12.5 μl of QuantiTect SYBR green RT-PCR master mix, 1 μl of template RNA, and 9.5 μl of nuclease-free water. The oligonucleotide primers for the PCR amplification are listed in Table 2 (3, 12). Each sample was placed in triplicate on a 96-well plate and subjected to one-step reverse transcription at 50°C for 30 min, 40 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 30 s and extension at 72°C for 30 s. Forty cycles of qRT-PCR are recommended in the Applied Biosystems 7500/7500 Fast real-time PCR getting starting guide for relative standard curve and comparative threshold cycle (C_T) experiments. The C_T means of almost all samples in our study were less than 40.

The relative expression level of efflux pump genes was calculated by the level of relative quantification in each efflux pump gene divided by that of *gapA*, a housekeeping gene, and then the expression rate of each efflux pump gene was defined as the relative expression level of the efflux pump gene in each isolate divided by that of the control (*E. coli* ATCC 25922) (3, 26).

Correlation of overexpression of efflux pump genes with MICs of each antibiotic. The overexpression of efflux pump genes was defined as ≥2-fold gene expression compared to that of the standard strain *E. coli* (ATCC 25922) in qRT-PCR (12). We analyzed the correlation of the overexpression of efflux pump genes with the MICs of each antibiotic agent.

Correlation of overexpression of efflux pump genes with risk factors for antibiotic resistance. We examined the correlation of risk factors for antibiotic resistance (age > 50 years, gender, presence of underlying urinary tract disease [uncomplicated or complicated UTI], indwelling urinary catheter, previous use of fluoroquinolones or other antibiotics, hospitalization, and treatment in a urological department) with the overexpression of efflux pump genes. Complicated and uncomplicated UTI were classified by the presence (complicated) or absence (uncomplicated) of underlying urinary tract disease. The previous use of antibiotics was defined as the administration of an antibiotic for more than 48 h during the previous year (2, 25).

Classification of groups by MICs of each antibiotic. We classified all isolates into four groups according to LVX resistance and the presence of underlying urinary tract disease (complicated or uncomplicated UTI), and we examined the correlation between the overexpression of efflux pump genes and each group in this categorization.

Statistical analysis. Statistical analyses were performed by univariate and multivariate logistic regression using STATA (StataCorp LP, College Station, TX) with $P < 0.05$ considered to indicate statistical significance. In addition, we analyzed the difference of the median expression rate of the efflux pump genes in each group (group 1, LVX-resistant isolates from complicated UTI; group 2, LVX-resistant isolates from uncomplicated UTI; group 3, LVX-susceptible isolates from complicated UTI; and group 4, LVX-susceptible isolates from uncomplicated UTI) shown in the previous section by the Wilcoxon rank-sum test.

RESULTS

Susceptibility to each antibiotic. The results of the susceptibility testing of 12 kinds of antimicrobial agents are shown in Table 3. No isolate showed resistance to IPM or AMK in our study.

Analysis of quantification of RNA expression by qRT-PCR. Among the 64 isolates, 52 isolates (81.3%) showed the overexpression of *marA*, 23 isolates (35.9%) of *yhiU*, 17 isolates (26.6%) of *yhiV*, and 22 isolates (34.4%) of *mdfA*. The median quantified expression rate of *marA* was 25.82 (range, 0.0024 to 178.08), that of *yhiU* was 1.31 (0.07 to 780), that of *yhiV* was 0.91 (0.1 to 32.38), and that of *mdfA* was 1.40 (0.05 to 83.87) (data not shown).

Correlation of overexpression of efflux pump genes with risk factors. We demonstrated significant correlations between the more than 2-fold overexpression of efflux pump genes and risk factors, especially the relationship between the overexpression

TABLE 2. Primer sequences used in this study

Regulator or type of transporter[a]	Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
reg	*marA*	AAACCGGTCATTCATTAGGC	GTATTTATGCGGCGGAACAT
MF	*mdfA*	CGGCAACGATATGATTCAAC	CAGTGACAGTTTCTCGCCTA
RND	*yhiU*	AATGTCACCTCGCCGATTAC	TGCCCTGAACCTGTTTGATT
RND	*yhiV*	CCGTACCGGTGGTTATTCTC	ATCGATTTATGCGTCGCTTC
Housekeeping gene	*gapA*	ACTTCGACAAATATGCTGGC	CGGGATGATGTTCTGGGAA

[a] reg, regulator; MF, major facilitator; RND, resistance nodulation division.

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TABLE 3. Susceptibilities of antimicrobial agents tested

Antibiotic	MIC range examined (μg/ml)	No.[a] (%) of isolates (n = 64)		
		Susceptible	Intermediate	Resistant
PIP	<4–>128	26 (40.6)	6 (9.4)	32 (50.0)
CFZ	<2–>64	41 (64.1)	4 (6.2)	19 (29.7)
CAZ	<2–>64	60 (93.8)	2 (3.1)	2 (3.1)
FEP	<2–>64	58 (90.6)	0 (0)	6 (9.4)
AMK	<4–>128	64 (100)	0 (0)	0 (0)
GEN	<1–>32	52 (81.3)	0 (0)	12 (18.7)
IPM	<1–>32	64 (100)	0 (0)	0 (0)
MIN	<1–>32	55 (85.9)	3 (4.7)	6 (9.4)
NAL	<0.06–>128	17 (26.6)	0 (0)	47 (73.4)
CIP	<0.015–>32	26 (40.6)	0 (0)	38 (59.4)
LVX	<0.015–>32	26 (40.6)	0 (0)	38 (59.4)
STX	<0.008–>8	26 (40.6)	0 (0)	38 (59.4)

[a] Susceptibility testing was performed according to Clinical Laboratory Standards Institute (CLSI) guideline M7-A8.

of *marA* with female gender or the previous use of fluoroquinolones, as shown in Table 4. In addition, we revealed a significant correlation between low-level resistance, more than 2- to 40-fold overexpression of *marA*, and female gender (odds ratio [OR] = 5.70; P = 0.05), and between high levels of resistance, more than 40-fold overexpression of *marA*, and patients treated in the urological department (OR = 32.7; $P <$ 0.01) by multivariate analyses. On the other hand, statistical analyses of high levels of resistance (more than 40-fold overexpression) for *yhiV*, *yhiU*, and *mdfA* could not be evaluated because of the very few cases (0 to 1) isolated in this study.

Correlation of overexpression of efflux pump genes with MICs of each antibiotic. We revealed a significant correlation of the overexpression of *marA* with higher MICs of FEP (OR, not applicable; $P < 0.01$), NAL (OR = 1.01; $P < 0.01$), CIP (OR = 1.05; P = 0.02), LVX (OR = 1.10; P = 0.03), and STX (OR = 4.31; P = 0.03) and of the overexpression of *mdfA* with higher MICs of LVX (OR = 1.04; P = 0.04) and STX (OR = 1.78; P = 0.02) by univariate analyses (data not shown). In addition, we demonstrated by multivariate analyses a significant relationship between the overexpression of (i) *marA* and higher MICs of FEP (OR > 0.18; P, not significant) or NAL (OR = 1.01; P = 0.03), (ii) *yhiV* and higher MICs of MIN (OR = 1.26; P = 0.03), and (iii) *mdfA* and higher MICs of STX (OR = 1.95; P = 0.03) (Table 5).

Classification of overexpression of efflux pump genes with each group. In our study, there were 38 strains (59.4%) that showed resistance to LVX and 45 strains (70.3%) isolated from complicated UTI patients. Twenty-nine isolates (45.3%) were classified as group 1 (LVX-resistant isolates from complicated UTI), 9 isolates (14.1%) as group 2 (LVX-resistant isolates from uncomplicated UTI), 16 isolates (25.0%) as group 3 (LVX-susceptible isolates from complicated UTI), and 10 isolates (15.6%) as group 4 (LVX-susceptible isolates from uncomplicated UTI). In these categories, we examined the expression level of the efflux pump genes in each group (Table 6 and Fig. 1) and demonstrated a significant correlation of the overexpression of *marA* with LVX-resistant isolates from complicated UTI (OR > 30.3; $P < 0.01$) and of the overexpression of *yhiU* with LVX-susceptible isolates from complicated UTI (OR = 8.24; P = 0.03) (Table 7), even though these categories had a small number of isolated bacteria.

DISCUSSION

Antibiotic resistance in *E. coli*, one of the most common pathogens in UTIs, has been encountered worldwide (17, 23). The emergence of multidrug resistance (MDR) in *E. coli*, such as ESBL-producing strains is disturbing (8, 16, 17), and the infections caused by MDR strains often are difficult to treat, especially in hospitalized patients (11). It is necessary to investigate prospectively the molecular mechanisms by which *E. coli* strains acquire drug resistance to prevent the further spread of these kinds of resistant strains or the occurrence of new resistant strains. Efflux pumps are found among all major categories of bacterial membrane transporters, and increased levels of the efflux system are considered to lead to MDR (3, 12, 24). The resistance nodulation division (RND) efflux pumps such as AcrAB-TolC and YhiUV and major facilitator superfamily (MFS) pumps such as MdfA play a major role in the efflux system of *E. coli* (22). Plasmid-mediated mechanisms, amino acid mutations of target enzymes for antibiotic agents, and antimicrobial-modifying resistance enzymes also were reported to contribute to MDR (4, 13, 17, 31).

The transcriptional activator MarA previously was shown to upregulate the production of the AcrAB efflux pump in *E. coli* (1). The investigation of the increased expression levels of MarA by qRT-PCR analysis showed that *marA* overexpression is a key factor leading to the overproduction of the AcrAB

TABLE 4. Patients' characteristics and correlation of overexpression of efflux pump genes with risk factors

Risk factor	No. (%) of patients	Correlation of risk factor with overexpression of indicated gene							
		marA		*yhiU*		*yhiV*		*mdfA*	
		OR	P	OR	P	OR	P	OR	P
Age > 50 yr	55 (85.9)	0.78	0.84	0.05	<0.01	0.42	0.39	1.06	0.95
Gender (female)	50 (78.1)	8.23	0.03	0.53	0.41	0.33	0.17	0.42	0.2
Urinary tract underlying disease	45 (70.3)	6.2	0.08	0.27	0.11	0.41	0.33	1.59	0.58
Patients treated in urological department	35 (54.7)	2.18	0.48	0.35	0.26	0.28	0.17	2.37	0.32
Previous use of other antibiotics	28 (43.8)	1.3	0.75	2.23	0.25	0.91	0.89	1.22	0.73
Hospitalization	22 (34.4)	1.08	0.93	0.22	0.1	0.54	0.47	2.14	0.31
Indwelling catheter	11 (17.2)	2.15	0.52	2.71	0.23	10.2	0.01	2.95	0.17
Previous use of fluoroquinolones	9 (14.1)	>16.6[a]	<0.01[a]	1.47	0.65	2.06	0.45	2.57	0.25

[a] All isolates that showed the overexpression of *marA* involved the previous use of fluoroquinolones.

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TABLE 5. Correlation of the overexpression of the efflux pump genes with MICs of each antibiotic by multivariate analyses[c]

Antibiotic	Correlation of antibiotic MIC with overexpression of indicated gene							
	marA		*yhiU*		*yhiV*		*mdfA*	
	OR	*P*	OR	*P*	OR	*P*	OR	*P*
PIP	1.01	0.06	1	0.22	1	0.83	1	0.41
CFZ	1.01	0.42	0.99	0.42	1	0.6	1	0.75
CAZ	1.07	0.64	0.67	0.31	0.72	0.37	1	0.81
FEP	>0.18[a]	NS[a]	0.98	0.36	0.99	0.42	0.99	0.46
AMK	1.06	0.8	0.9	0.54	0.99	0.98	0.97	0.84
GEN	1.04	0.2	0.95	0.07	0.99	0.51	1	0.51
IPM	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
MIN	0.94	0.17	1.03	0.46	1.26	0.03	0.99	0.78
NAL	1.01	0.03	1	0.31	1	0.76	1	0.22
CIP	1.04	0.06	1	0.5	0.99	0.43	1.02	0.18
LVX	1.08	0.08	1.01	0.58	1.02	0.52	1.05	0.08
STX	3.33	0.07	1.05	0.85	1.44	0.15	1.95	0.03

[a] All strains without overexpression of marA showed lower MICs of FEP.
[b] There was no IPM-resistant isolate.
[c] NA, not applicable; NS, not significant.

multidrug efflux pump in *E. coli* (12). MarA also was reported to correlate with fluoroquinolone resistance (22). Our study revealed that the overexpression of *marA* leads to higher MICs of CIP, LVX, and STX by univariate analyses and higher MICs of NAL by multivariate analyses, indicating that the overexpression of *marA* correlates with fluoroquinolone resistance. The overexpression of the efflux pump genes was reported to be one of the causes of the emergence of MDR bacterial isolates (22, 24). Our data indicated that the *marA* gene was the most important for LVX resistance in four kinds of efflux pump genes, especially in complicated UTI cases, and the overexpression of *marA* was correlated with the female gender. We also demonstrated an inverse correlation of the overexpression of *marA* with the male gender (OR = 0.12; $P < 0.01$). To our knowledge, there has been no report that demonstrated the correlation between gender and the overexpression of efflux pump genes. However, Tam et al. reported that there was a significant correlation between female gender and carbapenem-resistant *Pseudomonas aeruginosa* (30). We suggest that the expression level of the AcrAB efflux pump that was overproduced by the MarA transcriptional activator may differ by gender. Females may have more opportunity for UTI, regardless of the use of fluoroquinolones or its severity, than do males, meaning that females receive more antibiotic treatment than males (5). This might be one reason for resistance to these antibiotics.

YhiUV has a significant homology to AcrB but usually is expressed at a low level in clinical isolates (3). The YhiUV efflux system relates to decreased susceptibility to steroids and erythromycin (7, 19, 20) and is under the control of EvgAS, the regulator of the two-component signal transduction system (14, 20, 21). Bohnert et al. demonstrated the increased expression of *yhiUV* by qRT-PCR in two mutants of *E. coli*. The MICs of antibiotics such as fluoroquinolones, linezolid, and tetracycline were ≥2-fold increased for these isolates, and the MICs of macrolides such as azithromycin and telithromycin were decreased ≥2-fold (3). Our data on *yhiV* and antibiotic resistance demonstrated that the overexpression of *yhiV* contributes to resistance to MIN, suggesting that the YhiUV efflux system contributes to cross-resistance to tetracyclines and macrolides as mentioned above. We showed that the overexpression of *yhiU* is related to LVX-susceptible isolates in complicated UTI, even though previous reports showed that the overexpression of the YhiUV efflux pump was related to resistance to fluoroquinolones, as mentioned above (3, 12), indicating that there are regional and national geographic differences in bacterial characteristics. Rossi et al. also demonstrated a regional difference in antibiotic resistance in *E. coli* urinary isolates in a surveillance program for antimicrobial resistance (28). We demonstrated that the overexpression of *yhiU* and *yhiV* did not contribute to LVX resistance in complicated UTI cases, indicating that the YhiUV efflux pump is

TABLE 6. Expression of the efflux pump genes by each group classified by LVX resistance and underlying urinary tract disease[a]

Group	No. of isolates	Range (median) of expression			
		marA	*yhiU*	*yhiV*	*mdfA*
1	29	11.02–829.26 (67.02)	0.07–11.78 (0.76)	0.1–10.84 (0.66)	0.05–14.56 (1.66)
2	9	0.0024–3,178.08 (28.05)	0.33–27.83 (2.11)	0.11–32.38 (1.17)	0.07–83.87 (1.54)
3	16	0.14–156.93 (13.61)	0.42–780 (2.55)	0.25–8.63 (1.25)	0.41–18.57 (1.38)
4	10	0.29–25.03 (3.85)	0.25–13.61 (1.59)	0.18–5.63 (1.09)	0.18–7.2 (0.86)
Total	64	0.0024–3,178.08 (25.82)	0.07–780 (1.31)	0.1–32.38 (0.91)	0.05–83.87 (1.40)

[a] Group 1, LVX-resistant isolates from complicated UTI; group 2, LVX-resistant isolates from uncomplicated UTI; group 3, LVX-susceptible isolates from complicated UTI; and group 4, LVX-susceptible isolates from uncomplicated UTI.

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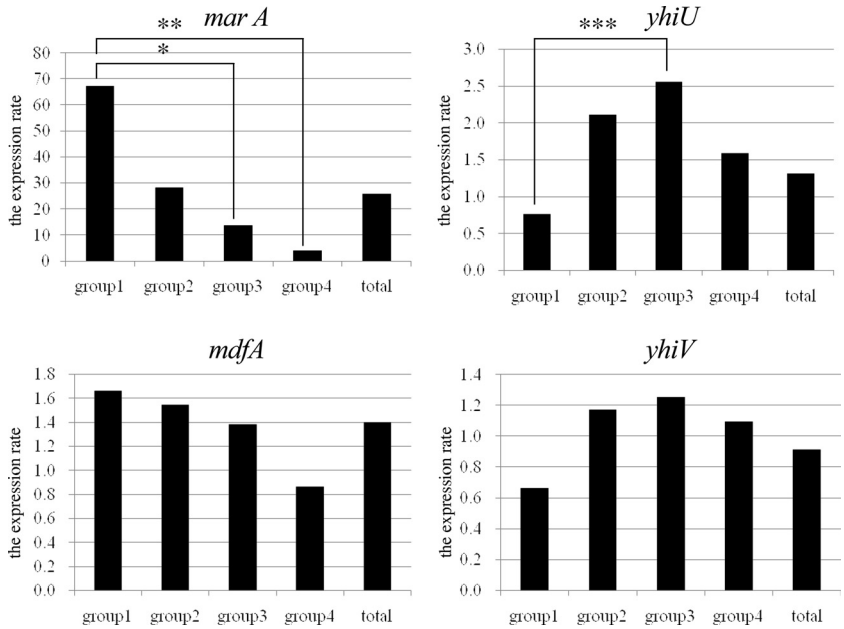

FIG. 1. Median expression rate of the efflux pump gene's mRNA categorized by LVX resistance and the presence of underlying urinary tract disease. These data are calculated from the relative expression level divided by that of the control, as shown in Materials and Methods. Groups were defined as LVX-resistant isolates from complicated UTI (group 1), LVX-resistant isolates from uncomplicated UTI (group 2), LVX-susceptible isolates from complicated UTI (group 3), and LVX-susceptible isolates from uncomplicated UTI (group 4), respectively. The *y* axis of each graph represents the expression rate of each efflux pump gene. Note that the median expression rate of the efflux pump genes demonstrated significant differences, which were indicated with asterisks (*, *P* value = 0.002; **, *P* value = 0.001; ***, *P* value = 0.004), in which the expression rate of *marA* and *mdfA* in group 1 was higher than that in group 4. More isolates showed the overexpression of *yhiU* and *yhiV* in group 3 than in group 1.

not as essential for fluoroquinolone resistance as the AcrAB pump, even though this group has a small number of bacterial strains.

MdfA is a multidrug transporter belonging to the MFS and is related to quinolone resistance (22). The overproduction of AcrAB and MdfA was reported to lead to an 8-fold-increased resistance to CIP (33). Our data on the overexpression of *mdfA* for higher MICs of LVX and STX is supported by their results. Our data correlating the *mdfA* efflux pump with resistance to STX appears to be a novel finding.

The characterization of the efflux system allows us to define the potential clinical roles of EPIs such as 1-(1-naphthylmethyl)-piperazine (6) and other EPIs that inhibit the AcrAB-TolC efflux system (11, 24). EPIs induce a significant reduction in resistance to antibiotics to which these isolates initially were resistant (24), indicating that the development of new EPIs would be a meaningful approach to the treatment of infections caused by MDR bacterial strains.

In conclusion, we demonstrated a relationship between the overexpression of *marA* and *mdfA* efflux pumps and resistance to NAL and STX in *E. coli* from UTI patients and showed a significant correlation between the AcrAB-TolC and MdfA efflux systems and resistance to fluoroquinolone. These findings contribute to the further understanding of the molecular mechanisms of efflux pump systems and how they contribute to antibiotic resistance and suggest that developing new EPIs could lead to new treatments for antibiotic-resistant bacteria.

TABLE 7. Correlation of overexpression of the efflux pump genes with each group by multivariate analysis[a]

Group	*marA*		*yhiU*		*yhiV*		*mdfA*	
	OR	*P*	OR	*P*	OR	*P*	OR	*P*
1	>30.3[b]	<0.01[b]	0.12	0.03	0.45	0.44	1.7	0.5
2	2.93	0.34	2.85	0.33	1.18	0.89	6.2	0.18
3	<0.01	<0.01	8.24	0.03	2.22	0.44	0.59	0.5
4	0.34	0.34	0.35	0.33	0.85	0.89	0.16	0.18

[a] Group 1, LVX-resistant isolates from complicated UTI; group 2, LVX-resistant isolates from uncomplicated UTI; group 3, LVX-susceptible isolates from complicated UTI; and group 4, LVX-susceptible isolates from uncomplicated UTI.

[b] All strains in group 1 demonstrated the overexpression of *marA*.

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