Fosfomycin (FOM) is an antibiotic produced by Streptomyces fradiae (1) and was approved for clinical use in Japan in 1980. FOM blocks MurA, which mediates bacterial peptidoglycan biosynthesis in its early step, showing a broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. FOM penetrates into bacterial cells via sugar transporters, such as GltP and UhpT, located at the cytoplasmic membrane, and spontaneous FOM-resistant mutants appear due to a reduction or lack of these transporters. Moreover, several enzymes, such as FosA, FosB, FosC, FosD, FomX, FomA, and FomB, have been reported, and FosA was first characterized as a glutathione S-transferase of FOM (2) (Fig. 1). After our first report about FosA3 and FosC2 in 2010 (3), FosA3-producing Escherichia coli isolates were recovered from humans, livestock, and/or pets (4–7), and the fosA3 gene has already transferred to Klebsiella pneumoniae (6) by a probable IS26 composite transposon carrying fosA3.

Acinetobacter soli HK001 was isolated from a blood culture of an infected human, and it showed very high resistance to FOM (MIC, >8,000 μg/ml) according to the agar dilution method recommended by the CLSI (8) in the presence of glucose-6-phosphate (G6P) (25 μg/ml), which induces UhpT. Four amplicons of class 1 integrons were found by PCR using 2 primers, 5’CS-
TABLE 1 MICs of fosfomycin for *A. soli* HK001 and *E. coli* DH10B transformed with the *fosK* gene

<table>
<thead>
<tr>
<th>Strain</th>
<th>FOM MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter soli</em> HK001</td>
<td>&gt;8,192</td>
</tr>
<tr>
<td><em>E. coli</em> DH10B</td>
<td>1</td>
</tr>
<tr>
<td><em>E. coli</em> DH10B(pBCSK+)</td>
<td>2</td>
</tr>
<tr>
<td><em>E. coli</em> DH10B(pBCSK+::fosK)</td>
<td>&gt;2,048</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>2</td>
</tr>
</tbody>
</table>

*FOM, fosfomycin. MICs were measured by the agar dilution method recommended by the CLSI.*

Class1-integron (5'-GGCATCAGCACAGAAG-3') and 3'CS-Class1-integron (5'-AAGCAGACTTGACCTGA-3'). An ampli- con of 1.2 kb was excised and purified. Its nucleotide sequence was
directly determined and revealed an *aacA4* gene and a new gene
case owner between *intI1* and the 3'CS (conserved se-
quence). The new case encoded a protein with a significant sim-
ilarity to other Fos proteins (Fig. 1) and was named FosK. The
deduced amino acid sequence of FosK showed 81% identity in its
amino acid sequence to open reading frame 1 (ORF1) of *Pseudomo-
as aeruginosa* (9). Moreover, 52%, 52%, 51%, 50%, 48%, and
47% amino acid identities were observed between FosK and
FosC2, FosD, FosA3, FosA, FosA2, and FosC, respectively, sug-
gest their close phylogenic relationship (Fig. 1). The *fosK* gene
was again amplified by PCR using total bacterial DNA and a
high-fidelity DNA polymerase, PrimeSTAR HS (TaKaRa Bio
Inc., Ohtsu, Japan), together with primers F2-BamHI (5'-CG
GGATCCGACATGGTTCAAACACGCCAGGC-3') and R2-
HindIII (5'-TACCCAAGCTTGGTCTTTGGGGCGGACTTGTA
GC-3'). The amplicon was ligated with pBCSK+ and cleaved by
BamHI and HindIII, and *E. coli* DH10B was transformed with the
recombinant plasmids. Then FOM-resistant transformants were
selected. After nucleotide sequencing of the insert on both strands,
a clone carrying no mutation in the *fosK* gene was finally chosen.
The FOM MIC for the transformant harboring intact *fosK* was
augmented to >2,048 µg/ml from 1 µg/ml for the recipient with
G6P (25 µg/ml) (Table 1).

FOM was recently considered to be a potent agent for treat-
ment of infections caused by multidrug-resistant bacteria, such as
extended-spectrum β-lactamase (ESBL)-producing *E. coli* and *K.
pneumoniae* (10). FOM has also been approved for veterinary
use in various countries (11). The *fosK* gene, together with *aacA4*,
is mediated by a class 1 integron, and thus this genetic element will
be further transmitted into various *Enterobacteriaceae*. Since FosK
confers on bacteria a very high level of resistance to fosfomycin, we
should diligently monitor the prevalence and trend of *fosK* as well
as of *fosA3* in both human and animals going forward.

**Nucleotide sequence accession number.** The *fosK* gene has
been assigned accession number AB917040.

**ACKNOWLEDGMENT**

This study was supported by grant no. H24-Shinko-Ippan-010.

**REFERENCES**

1. Rogers TO, Birnbaum J. 1974. Biosynthesis of fosfomycin by *Streptomy-
/10.1128/AAC.5.2.121.

protein (FosA) is a manganese metallo glutathione transferase related to
http://dx.doi.org/10.1021/bi963172a.

ience of fosfomycin resistance among CTX-M-producing *Escherichia coli*
clinical isolates in Japan and identification of novel plasmid-mediated
http://dx.doi.org/10.1128/AAC.01834-09.

of the plasmid-encoded fosfomycin resistance gene *fosa3* in *Escherichia coli*
dx.doi.org/10.1093/jac/dks465.

resistance region containing blaCTX-M-65 fosA3 and *rmtB* on conjugative
IncFI plasmids in *Escherichia coli* ST117 isolates from chicken. J. Med.

ience of acquired fosfomycin resistance among extended-spectrum
β-lactamase-producing *Escherichia coli* and Klebsiella pneumoniae clinical
isolates in Korea and IS26-composite transposon surrounding *fosa3*. J.

detection of fosfomycin resistance gene *fosa3* in CTX-M-producing *Esch-

antimicrobial susceptibility testing of bacteria that grow aerobically. Ap-
proved standard, 8th ed. Document M7-A9. Clinical and Laboratory Stan-
dards Institute, Wayne, PA.

The *ORF1* gene located on the class-1-integron-associated gene cassette
actually represents a novel fosfomycin resistance determinant. Antimi-

fosfomycin for treatment of urinary tract infections due to multidrug-
http://dx.doi.org/10.1128/AAC.00402-12.

11. Soraci AL, Perez DS, Martinez G, Diequez S, Tapia MO, Amanto F,
Harkes R, Romano O. 2011. Disodium-fosfomycin pharmacokinetics and