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Novel Integron-Mediated Fosfomycin Resistance Gene *fosK*

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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 GlpT 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 IS*26* 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-

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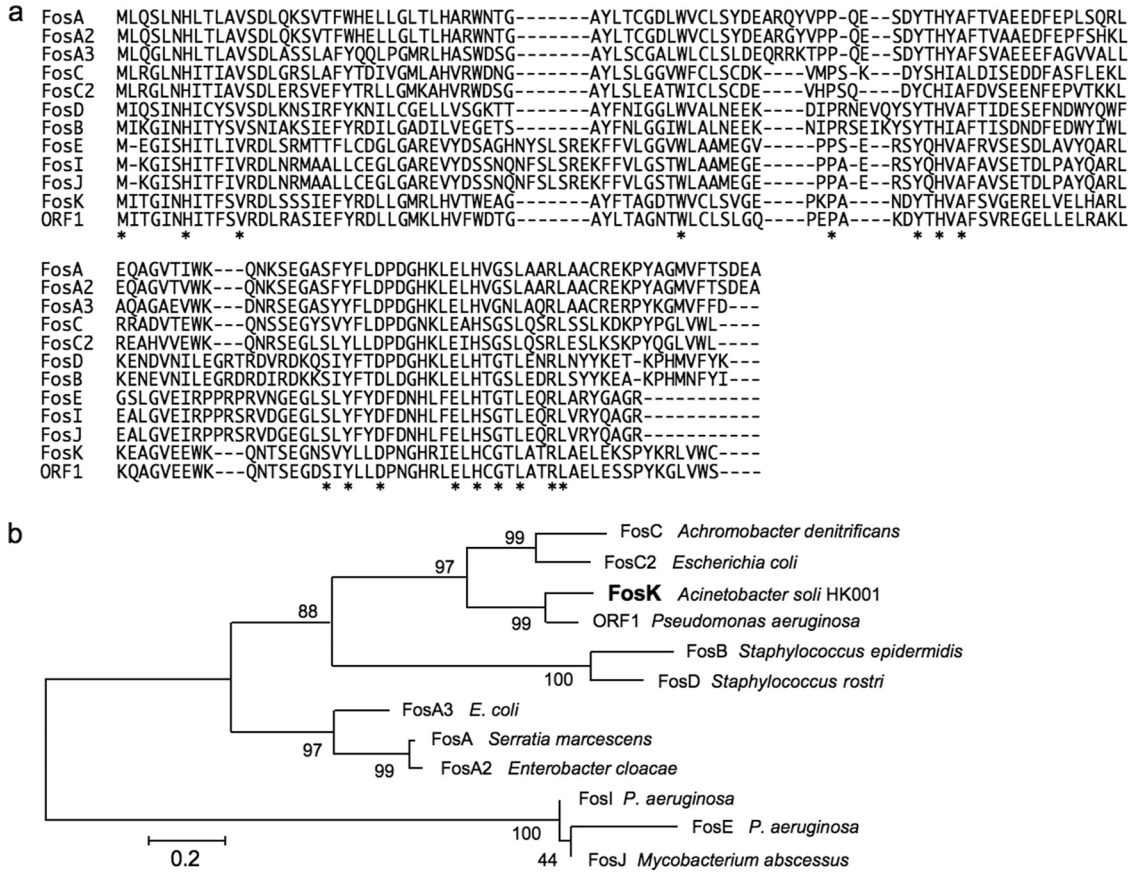

FIG 1 (a) Predicted amino acid sequences of FosK and other fosfomycin-modifying enzymes. *, amino acid residue conserved among the 12 fosfomycin resistance determinants. (b) Phylogenic relationships among the 12 glutathione *S*-transferases, including probable ones calculated by MEGA 5 (http://www.megasoftware.net/). GenBank or Protein Data Bank accession numbers are indicated for the following proteins: FosA (AAA98399), FosA2 (ACC85616), FosA3 (AB522970), FosB (CAA38136), FosC (AAZ14834), FosC2 (AB522969), FosD (AHB87392), FosE (BAO48025), FosI (BAO47999), FosJ (YP_006316014), FosK (AB917040), and ORF1 (AAP50248).

TABLE 1 MICs of fosfomycin for *A. soli* HK001 and *E. coli* DH10B transformed with the *fosK* gene

Strain	FOM MIC (μg/ml)[a]
Acinetobacter soli HK001	>8,192
E. coli DH10B	1
E. coli DH10B(pBCSK+)	2
E. coli DH10B(pBCSK+::*fosK*)	>2,048
E. coli ATCC 25922	2

[a] FOM, fosfomycin. MICs were measured by the agar dilution method recommended by the CLSI.

Class1-integron (5′-GGCATCCAAGCAGCAAG-3′) and 3′CS-Class1-integron (5′-AAGCAGACTTGACCTGA-3′). An amplicon of 1.2 kb was excised and purified. Its nucleotide sequence was directly determined and revealed an *aacA4* gene and a new gene cassette located between *intI1* and the 3′-CS (conserved sequence). The new cassette encoded a protein with significant similarity 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 *Pseudomonas 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, suggesting 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 GGATCCCGACATGGTTCAAACACGCCAGGC-3′) and R2-HindIII (5′-TACCCAAGCTTGGGTTTTGGGGCGGACTTGTA 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 treatment 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.

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