The increasing incidences of carbapenem-resistant bacteria, which are frequently resistant to most antibiotics, have renewed interest in revisiting the clinical use of old antibiotics, such as fosfomycin, for treating infections (1–3). Fosfomycin remains active against most Enterobacteriaceae isolates, with low-level resistance mediated most often by mutations in chromosomal loci, including glpT (4), muraA (3), and so on. Recently, two novel plasmid-encoded fosfomycin-inactivating enzymes, FosA3 and FosC2, were found among CTX-M-producing Escherichia coli isolates in Japan, China, and South Korea (5–7). In the majority of isolates expressing these enzymes, the corresponding genes are located on an IS26-flanked composite transposon inserted into the vicinity of blaCTX-M.

The most prevalent class A carbapenemase in clinical Enterobacteriaceae isolates are the Klebsiella pneumoniae carbapenemase (KPC) enzymes, and the KPC-2 variant is the most common (8). In Europe and the United States, blaKPC-2 is frequently located within a Tn3-based transposon, Tn4401 (8, 9). Similarly, Shen et al. (10) showed that the majority of carbapenem-resistant isolates, obtained from six eastern cities in China, carried blaKPC-2 within related but more complex chimeric elements. They comprise Tn1721, Tn3, Tn4401, and ISKpn8 fragments and a segment similar to the plasmid RA3. This complex composite transposon possesses Tn1721-derived termini that match the intact transposase gene and an internal blaKPC-2-bearing 2-kb fragment showing significant sequence similarity to Tn4401 (10). In this study, we report the coexistence of fosA3 and blaKPC-2 on a single transposon found within a conjugative IncP plasmid, which was identified in two temporally and spatially related clinical isolates belonging to distinct Enterobacteriaceae species. To our knowledge, this is the first report of a transposon carrying the fosfomycin resistance gene fosA3 and the carbapenemase gene blaKPC-2.

**MATERIALS AND METHODS**

**Bacterial strains and antibiotic susceptibility testing.** Two clinical isolates, of E. coli strain HS102707 and Enterobacter aerogenes strain HS112625, were collected after they were found to not be sensitive to imipenem and fosfomycin in routine antibiotic susceptibility testing. Among 78 blaKPC-positive clinical K. pneumoniae isolates for which we sequenced the genetic environment of blaKPC-2, strain HS092839 has a blaKPC-2 environment similar to that of E. coli strain HS102707 or E. aerogenes strain HS112625; that is, a similar blaKPC-2-carrying transposon was inserted into a similar 70-kb plasmid. Therefore, the K. pneumoniae HS092839 isolate was also included in this study.
The MICs of amikacin, ceftazidime, cefotaxime, imipenem, ertapenem, meropenem, piperacillin-tazobactam, and fosfomycin were determined by the Etest technique (AB Biodisk, Sweden) for the three isolates tested positive for KPC-2 by PCR, and Kp-39 were determined (Table 1), which confirms that all three isolates tested positive for KPC-2.

**RESULTS AND DISCUSSION**

Fosfomycin resistance is uncommon among *Enterobacteriaceae* clinical isolates. As part of a broader study focused on carbapenem-resistant *Enterobacteriaceae* isolates obtained at Huashan Hospital, Shanghai, from August 2006 to December 2011, two isolates, from *E. coli* strain HS102707 (Ec-07) and *E. aerogenes* strain HS112625 (Ea-25), which exhibited resistance to fosfomycin and intermediate resistance to imipenem, were chosen for further characterization. A third multidrug-resistant, fosfomycin-sensitive *Enterobacteriaceae* isolate from the same collection, *K. pneumoniae* strain HS092839 (Kp-39), carrying a similar plasmid and Tn1721 transposon structure, was also analyzed for comparison. The MICs of fosfomycin for the Kp-39, Ec-07, and Ea-25 transconjugants were 0.38 mg/liter, 48 mg/liter, and >1,024 mg/liter, respectively. All three isolates tested positive for *bla*KPC-2 by PCR, and Ec-07 and Ea-25 were also positive for *fosA3*.

The *E. coli* (Ec-07) and *K. pneumoniae* (Kp-39) isolates were also characterized by MLST and found to belong to ST46 and ST11, respectively, according to corresponding species-specific MLST schemes of Pasteur. Consistent with previous reports, ST11 has been shown to be a predominant *K. pneumoniae* sequence type identified among clinical isolates from China (15,16). In contrast, *E. coli* ST46 (or ST15 in Achtman MLST schemes) has not been reported to be a dominant clone in China or elsewhere (17–19).
**fosA3** and **bla_{KPC-2}** are carried on the same conjugative plasmid. In order to further explore the genetic basis of resistance, we performed conjugation experiments with each of the three primary isolates serving as a donor and the azide-resistant *E. coli* J53 as the recipient. Transconjugants were selected on medium containing ampicillin and azide. Next, electrophoretic plasmid profiles of the parental isolates were compared with those of matching transconjugants. Based on these data, it was evident that the parent isolate pairs differed in the number of plasmids they carried. The three *K. pneumoniae* isolates, *J53* HS112625, *E. coli* HS112625, and *E. aerogenes* HS112625 were digested with EcoRI; in lanes 2, 4, and 6, plasmids were extracted from *E. coli* J53 HS092839 transconjugant, and *E. coli* J53 HS112625 transconjugant, respectively. The asterisk in panel b indicates a faint **bla_{KPC-2}**-Positive band. (c) In lanes 1, 2, and 3, plasmids derived from isolates *K. pneumoniae* HS092839, *E. coli* HS112625, and *E. aerogenes* HS112625 were digested with HindIII; in lanes 4, 5, and 6, plasmids derived from isolates *K. pneumoniae* HS092839, *E. coli* HS112625, and *E. aerogenes* HS112625 were digested with HindIII.

This complex element was inserted in the stability module of pH5102707. Critically, consistent with its ready mobilization by conjugation, pH5102707 encodes a full complement of conjugation machinery, including a type IV secretion system (T4SS), a relaxase, and a cognate origin of transfer (oriT) sequence. The pH5102707 T4SS is encoded by the *trbA-trbP* and *traK-traM* gene clusters.

Approximately 50% of *Gammaproteobacteria* plasmids are potentially transmissible (conjugative and mobilizable) (23). Relaxase is the only component common to all transmissible plasmids (24, 25). Based upon putative protein sequence similarities, a total of 741 sequences of relaxase encoded by 673 plasmids have been taken from 1,730 plasmid sequences deposited in GenBank (25). All the plasmids with relaxase can be classified into one of six families (MOB_{P}, MOB_{V}, MOB_{R}, MOB_{B}, MOB_{C}, and MOB_{B}) and 31 subfamilies (23–25). MOB_{P} was the most represented (273 plasmids) by a degenerate primer MOB typing (DPMT) method (26). Within each family, subfamilies corresponding to phylogenetic clades contain more members (e.g., MOB_{B}) which harbors relaxases encoded by IncF plasmids, and MOBP11, which groups relaxases encoded by IncP plasmids (23). Phylogenetic analysis shows that the three relaxases encoded by pH5102707, pH5092839, and pH5112625 were grouped as the P1 relaxase subfamily (Fig. 2b). Relaxase protein sequence comparison of pH5102707 revealed 30.5% identity with RP4 (Inc P1), the prototype plasmid for the P11 subfamily. The relaxase most similar to that of pH5102707, pXFAS01, exhibited 47% identity; no identity was found with any other non-P11 subfamily relaxases by NCBI blast.

**The **bla_{KPC-2}** and **fosA3** genes are borne on a novel mosaic transposon.** The **fasA3** gene in pH5102707 is embedded in a variant of the recently described **bla_{KPC-2}**-bearing transposon found on pKP048 (Fig. 3). **bla_{KPC-2}** is located in a Tn1721-Tn3 chimeric element flanked by two 38-bp inverted repeat sequences, IRR and IRL2. In pH5102707 and pH5092839, Tn1721-Tn3 was inserted close to the gene klcA, but in pKP048, it was located next to a truncated IS26-tpaA. pKP048 belongs to the F family (IncF), while

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**FIG 1** Electrophoretic profiles of plasmids (a), hybridization with a **bla_{KPC-2}**-specific probe (b), and plasmids digested with EcoRI and HindIII (c). (a and b) In lanes 1, 3, and 5, plasmids were extracted from parental isolate *K. pneumoniae* HS092839, *E. coli* HS102707, and *E. aerogenes* HS112625, respectively; in lanes 2, 4, and 6, plasmids were extracted from *E. coli* J53 HS092839 transconjugant, *E. coli* J53 HS102707 transconjugant, and *E. coli* J53 HS112625 transconjugant, respectively. The asterisk in panel b indicates a faint **bla_{KPC-2}**-Positive band. (c) In lanes 1, 2, and 3, plasmids derived from isolates *K. pneumoniae* HS092839, *E. coli* HS112625, and *E. aerogenes* HS112625 were digested with EcoRI; in lanes 4, 5, and 6, plasmids derived from isolates *K. pneumoniae* HS092839, *E. coli* HS112625, and *E. aerogenes* HS112625 were digested with HindIII.
pHS102707, pHS092839, and pHS112625 belong to the P11 subfamily (IncP1). This suggests that a block mobilization of the complex transposon may happen among IncP1 plasmids. Interestingly, EcoRI and HindIII digestion profiles (Fig. 1c) show that the three P11 subfamily plasmids are not identical, and some differences exist.

The \( \text{bla}^{\text{KPC-2}} \)-flanking regions among pHS102707, pHS092839, and pKP048 on pHS102707 showed a high degree of synteny. However, several insertion and/or deletion events had taken place within the \( \text{Tn1721} \)-like region. \( \text{Tn1721} \) was intact in pKP048, carrying intact termini and transposase genes. In pHS092839, IS26 and a truncated \( \text{Tn21} \)-\( \text{tnpR} \) had been inserted into \( \text{Tn1721} \), resulting in the truncated \( \text{Tn1721} \)-\( \text{tnpA} \) and deletion of \( \text{Tn1721} \)-\( \text{tnpR} \). Remarkably, in pHS102707, a DNA fragment newly found in this study, IS26-\( \text{orf3} \)-\( \text{orf2} \)-\( \text{orf1} \)-\( \text{fosA3} \)-IS26-\( \text{Tn21} \)-\( \text{tnpR} \), had been inserted into \( \text{Tn1721} \)-\( \text{tnpA} \). The IS26 composite transposon in pHS102707 is the same as that found in \( \text{E. coli} \) isolated from livestock (GenBank accession no. JQ432559) (26).

The plasmid-borne \( \text{fosA3} \) fosfomycin resistance gene was first reported in \( \text{E. coli} \) isolates collected between 2002 and 2007 in Japan (7) and has since been reported in China and South Korea. Where characterized, \( \text{fosA3} \) has been found in an IS26-associated context on different plasmids from \( \text{E. coli} \) and \( \text{K. pneumoniae} \) (5, 26) and has frequently been linked physically to one of several \( \text{bla}^{\text{CTX-M}} \) variants and, occasionally, to the aminoglycoside resistance-encoding \( \text{rmtB} \) gene. Our findings, combined with those of the present study, suggest that the IS26 composite transposon is highly mobile, appearing in the plasmid harboring \( \text{bla}^{\text{CTX-M}} \) as well as the transposon with \( \text{bla}^{\text{KPC-2}} \). Additionally, it is interesting to note that the two IS26 insertions in pHS102707 and pHS092839 occurred in the exactly the same position as in pKP048. pHS102707 has an IS26-mediated composite transposon in comparison to pHS092839, highlighted here by the insertion of \( \text{fosA3} \). Since IS26 is often present in the vicinity of a long list of resistance genes, this insertion sequence is likely to contribute to the accelerated emergence of other elements carrying \( \text{fosA3} \) alongside various repertoires of preexisting resistance determinants.

In conclusion, we report from this study an IS26-flanked composite transposon which has mobilized \( \text{fosA3} \) onto an \( \text{bla}^{\text{KPC-2}} \)-bearing \( \text{Tn1721} \)-\( \text{Tn3} \)-derived mosaic transposon in an ST11-type \( \text{K. pneumoniae} \) isolate. This brings together genes coding for resistance to two classes of last-line antimicrobial agents on

FIG 2 (a) Schematic map of pHS102707, an IncP plasmid found in the carbapenem-resistant \( \text{E. coli} \) isolate investigated in this study. Genes shown in red, blue, green, and orange are involved in replication, stability, propagation, and adaptation, respectively. Genes encoding unknown functions or that are not directly related to the above-mentioned roles are indicated in gray and shown unlabeled. Red bars highlight \( \text{bla}^{\text{KPC-2}} \), \( \text{fosA3} \), and the relaxase gene (\( \text{traI} \)). \( \text{Tn1721} \)-specific inverted repeats that define the boundaries of the associated mosaic transposon are labeled IR1 and IR2. A third matching internal repeat sequence is labeled IR3. (b) Inferred phylogenetic relationships of the plasmid-coding relaxase homologs. Seventeen protein sequences were aligned and the tree was generated with MEGA5 (21, 22) using the maximum-likelihood method. The relaxase sequences obtained in this study are indicated by blank arrows, while others were taken from Alvarado et al. (25). The sequences marked by an asterisk denote the ones taken from the prototype plasmid of each subfamily.
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We declare no conflicts of interest.

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