AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Characterization of SXT/R391 Integrative and Conjugative Elements in *Proteus mirabilis* Isolates from Food-Producing Animals in China

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SXT/R391 integrative and conjugative elements (ICEs) were detected in 8 out of 125 *Proteus mirabilis* isolates from food-producing animals in China. Whole-genome sequencing revealed that seven ICEs were identical to ICE*Pmi*Jpn1, carrying the cephalosporinase gene bla_{CMY-2}. Another one, designated ICE*Pmi*Chn1, carried five resistance genes. All eight ICEs could be transferred to *Escherichia coli* via conjugation. The results highlight the idea that animal farms are important reservoir of the SXT/R391 ICE-containing *P. mirabilis*.

Integrative and conjugative elements (ICEs) are self-transmissible chromosomal mobile genetic elements (MGEs) that can bestow new traits on bacteria, such as increased virulence, biofilm formation, and resistance to antimicrobials and heavy metals (1), which have an important impact on bacterial evolution and can mediate the dissemination of antimicrobial resistance (1–4). SXT/R391 ICEs are chromosomal MGEs sharing a conserved integrase that could mediate site-specific integration into the 5′ end of *prfC* (5). This family of ICEs contains 52 nearly identical core genes, many of which are involved in integration/excision, conjugative transfer, and regulation of the ICEs (6, 7). In addition, five hot spots (HS1 to -5) and four variable regions (VRI to -IV) have been identified which contain variable genes conferring element-specific properties and providing their hosts with fitness functions (6, 8–10).

SXT/R391 ICEs have been found in several genera of *Gammaproteobacteria* (http://db-mml.sjtu.edu.cn/ICEberg/) (11), such as *Vibrio*, *Providencia*, *Proteus*, and some marine bacteria (3, 6, 9, 10). *Proteus mirabilis*, a member of the family *Enterobacteriaceae*, has become one of the most important opportunistic pathogens in nosocomial infections in China (12). Four SXT/R391 ICEs, R997 (India), ICE*Pmi*USA1 (United States), ICE*Pmi*Jpn1 (Japan and Spain), and ICE*Pmi*Spn1 (Spain), have been detected in *P. mirabilis* (3, 13–16). It is worth noting that the cephalosporinase gene bla_{CMY-2} is inserted into ICE*Pmi*Jpn1 and ICE*Pmi*Spn1 via IS*10*-mediated transposition (15, 16), indicating that SXT/R391 ICEs are the important mobile platforms to capture new resistance genes. However, data on the SXT/R391 ICEs in *P. mirabilis* isolates from China are still lacking.

In this study, a total of 125 *P. mirabilis* strains (64 from chicken and 61 from swine), which were isolated from the intestinal tracts of animals among 11 poultry and 35 swine farms in 16 provinces in China between March 2012 and December 2014, were screened for the presence of the integrase gene *int* of SXT/R391 ICEs and bla_{CMY-2} by PCR with primers listed in Table S1 in the supplemental material (9, 15, 17, 18). SXT/R391 ICEs and bla_{CMY-2} were detected in eight and 16 strains, respectively. The eight strains carrying SXT/R391 ICEs displayed multidrug resistance profiles, as determined by the disk diffusion method according to the CLSI guidelines (19) (Table 1), and seven of these also contained bla_{CMY-2}. The clonal relationships of the ICE-harboring strains were determined by pulsed-field gel electrophoresis (PFGE) analysis with genomic DNA digested by SmaI (Fig. 1). Two strains, Pm14C12 and Pm14C58, from different chicken farms in Sichuan Province had similar PFGE profiles, indicating the presence of clonal spread of ICE-harboring strains. The origin and antimicrobial resistance profiles of the eight ICE-harboring strains are listed in Table 1. To the best of our knowledge, this is the first report of SXT/R391 ICEs in *P. mirabilis* from China.

Whole-genome sequencing was performed on the Illumina MiSeq (Majorbio, Shanghai) using a 400-bp paired-end library with ~200-fold average coverage. The paired-end reads were assembled *de novo* using the SOAPdenovo v2.04 and GapCloser v1.12. The gaps among contigs were filled in by PCR linkage. The complete nucleotide sequences of SXT/R391 ICEs were analyzed by using the BLAST programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence analysis showed that two different SXT/R391 ICEs were present in the eight strains. Seven R391-like ICEs (91,091 bp) were identical to the ICE*Pmi*Jpn1, which carried the bla_{CMY-2} gene and was previously reported in Japan and Spain (15, 16). Given that the IS*10*-mediated transposition region carrying the bla_{CMY-2} gene was inserted into *S025* in ICE*Pmi*Jpn1 and ICE*Pmi*Spn1, we renamed this region as variable region V (Fig. 2). The nucleotide sequences of ICE*Pmi*Jpn1 reported in this study were almost 100% identical to the corresponding incomplete nucleotide sequences of ICE*Pmi*Jpn1 in Japan (15), with only a 2-bp difference in HS3. Our data showed that 43.75% (7 of 16) bla_{CMY-2} genes in *P. mirabilis* were carried by ICE*Pmi*Jpn1. Mata et al. also reported that 36.84% (7 of 19) bla_{CMY-2} genes in *P. mirabilis* clin-

Received 25 November 2015 **Returned for modification** 11 December 2015
Accepted 30 December 2015

Accepted manuscript posted online 11 January 2016

Citation Lei C-W, Zhang A-Y, Wang H-N, Liu B-H, Yang L-Q, Yang Y-Q. 2016. Characterization of SXT/R391 integrative and conjugative elements in *Proteus mirabilis* isolates from food-producing animals in China. Antimicrob Agents Chemother 60:1935–1938. doi:10.1128/AAC.02852-15.

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.02852-15.

TABLE 1 Origin, antimicrobial resistance profiles, and transconjugative frequencies of SXT/R391 ICEs of the eight *P. mirabilis* strains

Strain	Province of isolation	Date of isolation	Host	Antimicrobial resistance profile[a]	ICE type	Transconjugative frequency
PM12C07	Shandong	29 October 2012	Chicken	**AMX**, **AMC**, **FOX**, **CTX**, NAL, NOR, CIP, DOX, SXT	ICE*Pmi*Jpn1	5.5×10^{-5}
PM12C31	Chongqing	3 November 2012	Swine	**AMX**, **AMC**, **FOX**, **CTX**, DOX, STR, SPT, SXT	ICE*Pmi*Jpn1	2.3×10^{-5}
PM13C04	Hubei	16 April 2013	Chicken	AMX, NAL, NOR, **DOX**, **STR**, **SPT**, **SXT**, **FFC**	ICE*Pmi*Chn1	2.8×10^{-6}
PM13C23	Henan	27 November 2013	Chicken	**AMX**, **AMC**, **FOX**, **CTX**, NAL, NOR, CIP, DOX, SXT	ICE*Pmi*Jpn1	1.5×10^{-4}
PM14C12	Sichuan	5 March 2014	Chicken	**AMX**, **AMC**, **FOX**, **CTX**, NAL, NOR, DOX, STR, SPT, SXT	ICE*Pmi*Jpn1	2.5×10^{-5}
PM14C28	Heibei	22 April 2014	Chicken	**AMX**, **AMC**, **FOX**, **CTX**, NAL, NOR, CIP, DOX, SXT, FFC	ICE*Pmi*Jpn1	3.2×10^{-5}
PM14C39	Sichuan	29 April 2014	Swine	**AMX**, **AMC**, **FOX**, **CTX**, NAL, NOR, DOX, SXT, FFC	ICE*Pmi*Jpn1	1.9×10^{-7}
PM14C58	Sichuan	12 December 2014	Chicken	**AMX**, **AMC**, **FOX**, **CTX**, NAL, NOR, DOX, STR, SPT, SXT	ICE*Pmi*Jpn1	3.6×10^{-6}

[a] AMX, amoxicillin; AMC, amoxicillin-clavulanate; FOX, cefoxitin; CTX, cefotaxime; NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; DOX, doxycycline; STR, streptomycin; SPT, spectinomycin; SXT, trimethoprim-sulfamethoxazole; FFC, florfenicol. Resistance to the antimicrobials shown in bold is conferred by genes present on SXT/R391 ICE.

ical isolates from Spain were associated with SXT/R391 ICEs (16). The results indicated that SXT/R391 ICEs are the important MGEs for the dissemination of bla_{CMY-2} genes in *P. mirabilis*.

Strain PM13C04 contained a novel multidrug-resistant SXT-like ICE that was designated ICE*Pmi*Chn1 (92,751 bp), according to the nomenclature proposed by Burrus et al. (5). The ICE*Pmi*Chn1 belonged to the S exclusion group determined by the amino acid sequences of TraG and Eex proteins (20). The nucleotide sequences of *int* (1242 bp) in ICE*Pmi*Chn1 showed 97% identity to that of SXT and 96% identity to that of R391 (21, 22), which also varied from those of previously reported SXT/R391 ICEs according to the maximum likelihood tree obtained by using MEGA 6 software (23) (Fig. 3). ICE*Pmi*Chn1 harbored a multidrug resistance region in VRIII, containing five resistance genes, *floR*, *tet*(G), *strA*, *strB*, and *sul2*. ICE*Pmi*Chn1 shared HS1, HS2, and HS3 gene contents with ICE*Eni*SpaI from *Enterovibrio nigricans* (9), SXT (21) and R391 (22), respectively, indicating that ICE*Pmi*Chn1 might come from homologous recombination among different SXT/R391 ICEs (4, 6, 24). It was noticeable that two open reading frames (ORFs) (*chn1* and *chn2*) and the insertion sequence IS*Ppu12* in HS4 were reported here for the first time in a SXT/R391-related ICE (Fig. 2). *chn1* and *chn2* showed 93% and 92% nucleotide identity to the corresponding genes in *Vibrio* sp. EJY3 (CP003241). A protein BLAST search showed that *chn1* encodes the predicted SIR2 superfamily of protein (472 amino acids [aa]), including the silent information regulator 2 (Sir2) enzyme, which catalyzes NAD^+-dependent protein/histone deacetylation, and *chn2* encodes a predicted ATPase (679 aa). The insertion sequence IS*Ppu12* (3,372 bp), which was first characterized in *Pseudomonas putida* (25), was inserted into *s062*, leading to an 8-bp target site duplication (TAAAGAAA). IS*Ppu12* consists of four ORFs, *tnpA*, *lspA*, *orf1*, and *tnpR*. *orf1* encodes a possible divalent heavy metal/H^+ antiporter protein (26), which might provide fitness in the event of exposure to heavy metal. In HS5 five genes showed 97 to 98% nucleotide identity to the corresponding genes that encoded the type III restriction-modification system conferring resistance to phage infection in ICE*Val*SpaI from *Vibrio alginolyticus* (10), with the exception of the *met* gene, which showed only 71% nucleotide identity.

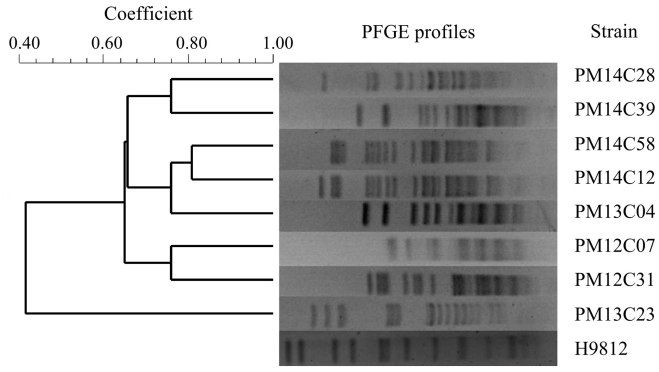

FIG 1 PFGE analysis of the eight *P. mirabilis* strains carrying SXT/R391 ICEs. PFGE analysis with SmaI digestion was carried out on CHEF Mapper under the following conditions: Auto Algorithm; 30 kb, low MW; 400 kb, high MW; 16.5 h. *Salmonella enterica* serovar Braenderup strain H9812 was used as a reference.

Most of the SXT/R391 ICEs could be excised from the chromosome and transferred to new hosts via conjugation (5). We detected the circular form of ICEs using the primers LE4 and RE4 (17). A 550-bp target band was detected in all eight ICE-harboring strains (see Fig. S1 in the supplemental material), indicating that the ICEs could form the circular intermediates. Conjugation experiments were performed by using the rifampin-resistant strain *Escherichia coli* EC600 as the recipient strain, with selection on agar plates containing 300 μg/ml rifampin and 4 μg/ml cefotaxime (or 8 μg/ml florfenicol and 16 μg/ml tetracycline together). The transconjugants were further determined by the mobilization of antimicrobial resistances and by PCR targeting the ICE *int* gene. The results confirmed that all eight ICEs could be transferred to *E. coli*, with transfer rates ranging from 1.5×10^{-4} to 1.9×10^{-7} transconjugants per recipient cell (Table 1), suggest-

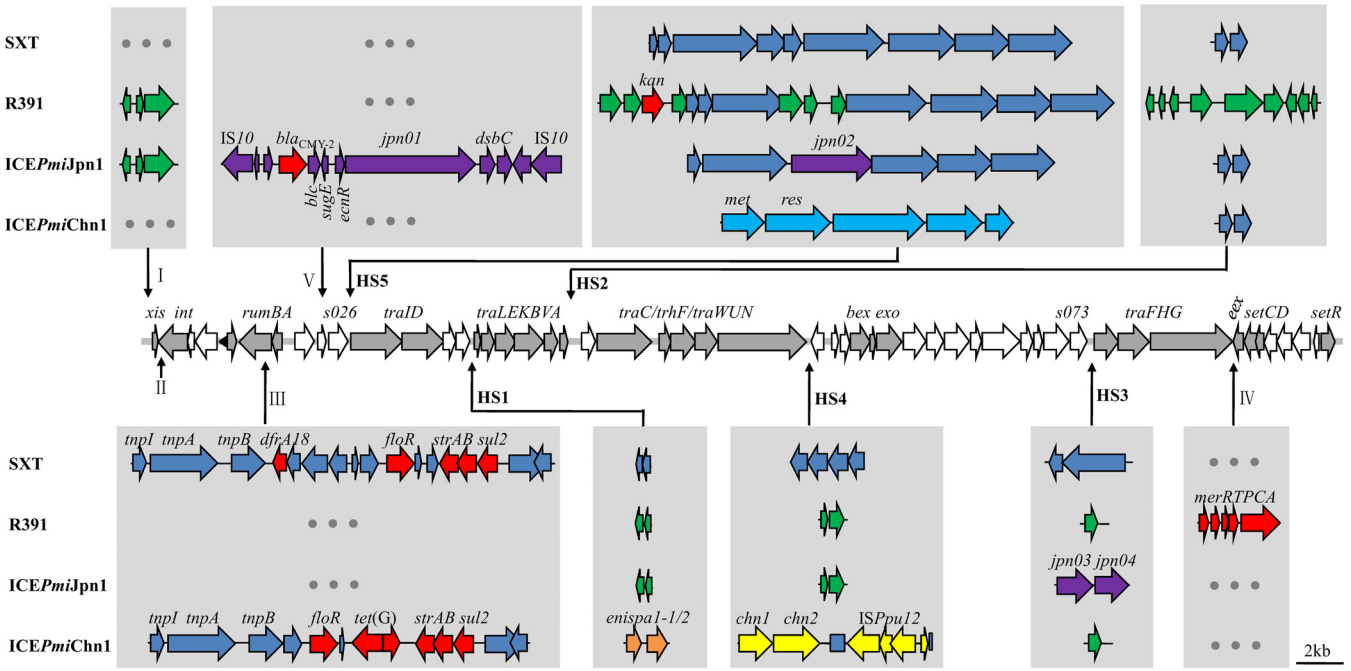

FIG 2 Schematic view of the two SXT/R391 ICEs, ICE*Pmi*Jpn1 and ICE*Pmi*Chn1, and comparison with SXT and R391. The line of arrows in the middle represents the 52 conserved core genes of the SXT/R391 family of ICEs. HS1 to HS5 represent hot spots 1 to 5, and I to V represent the variable regions I to V. Variable genes from originally described ICEs are shown with different colors: SXT (21), blue; R391 (22), green; ICE*Pmi*Jpn1, purple; ICE*Pmi*Chn1, yellow; ICE*Val*SpaI (10), light blue; ICE*Eni*SpaI (9), orange. Resistance genes are in red.

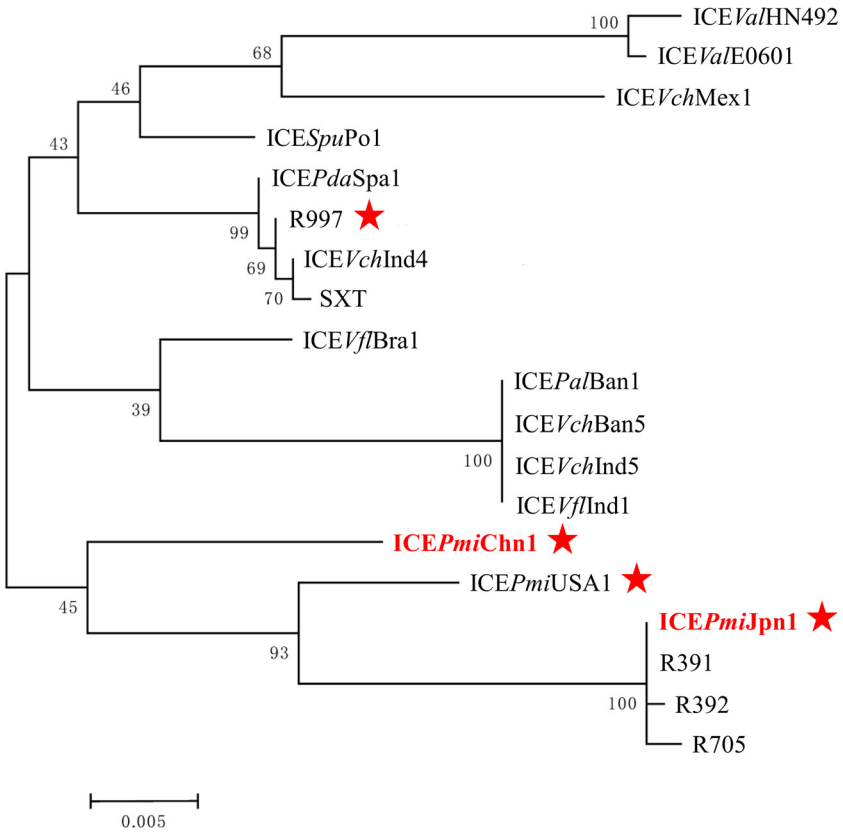

FIG 3 Maximum likelihood phylogenetic tree based on 19 *int* gene sequences from SXT/R391 ICEs by using MEGA 6. Bootstrap analysis was performed with 1,000 replications. The SXT/R391 ICEs from *P. mirabilis* are indicated by a red star. The *int* gene sequences reported in this study are in red, and others are in black and were downloaded from GenBank with the accession numbers listed in Table S2 in the supplemental material. Bar, 0.05 substitution per nucleotide position.

ing that the horizontal transfer might be an important mechanism for the dissemination of SXT/R391 ICEs.

In conclusion, this is the first report of SXT/R391 ICEs in *P. mirabilis* in China. The results highlight the idea that animal farms are important reservoirs of the *P. mirabilis* strains carrying SXT/R391 ICEs. A novel multidrug-resistant ICE, ICE*Pmi*Chn1, was characterized in *P. mirabilis* for the first time. SXT/R391 ICEs could mediate the dissemination of antimicrobial resistance in *P. mirabilis* and be transferred to other members of the *Enterobacteriaceae*, which should receive more attention.

Nucleotide sequence accession numbers. The nucleotide sequences of the complete ICE*Pmi*Jpn1 and ICE*Pmi*Chn1 characterized in this study were submitted to GenBank and assigned accession numbers KT894734 and KT962845, respectively.

ACKNOWLEDGMENT

We are very grateful to Wang Ming-Gui (Institute of Antibiotics, Huashan Hospital, Fudan University) for providing *E. coli* EC600.

FUNDING INFORMATION

This work was supported by "973" National Basic Research Program of China (project 2013CB127200), Earmarked Fund for Modern Agroindustry Technology Research System (grant CARS-41-K09), Special Fund for Agro-scientific Research in the Public Interest of China (grants 201303044 and 201403054), Science & Technology Pillar Program in Sichuan Province (grant 2013NZ0025), and the general program of National Natural Science Foundation of China (grants 31572547 and 31572548).

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