Molecular Characteristics of *Salmonella* Genomic Island 1 in *Proteus mirabilis* Isolates from Poultry Farms in China

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Six out of the 64 studied *Proteus mirabilis* isolates from 11 poultry farms in China contained *Salmonella* genomic island 1 (SGI1). PCR mapping showed that the complete nucleotide sequences of SGI1s ranged from 33.2 to 42.5 kb. Three novel variants, SGI1-W, SGI1-X, and SGI1-Y, have been characterized. Resistance genes *lnuF*, *dfrA25*, and *qnrB2* were identified in SGI1 for the first time.

Genomic islands are discrete DNA segments and have the capacity for integration into the chromosome of bacteria, influencing traits such as antibiotic resistance, symbiosis, fitness, and adaptation (1). *Salmonella* genomic island 1 (SGI1) is a 42.4-kb mobilizable element initially identified in the multidrug-resistant (MDR) *Salmonella enterica* serovar Typhimurium phage-type DT104 clone (2, 3). SGI1 consists of a backbone containing 28 open reading frames (ORFs) (S001 to S027 and S044) and an MDR region. The MDR region is a complex class 1 integron named In104 (4), containing five antibiotic resistance genes conferring resistance to ampicillin (Ap), chloramphenicol (Cm) and florfenicol (Ff), streptomycin (Sm) and spectinomycin (Sp), sulfonamides (Su), and tetracycline (Tc) (i.e., ApCmFfSmSpSuTc resistance phenotype) (3). SGI1 variants predominantly result from insertion sequence, homologous recombination, transposition, and loss or exchange of gene cassettes within the MDR region (5).

Proteus mirabilis, a Gram-negative member of the family *Enterobacteriaceae*, is recognized as an opportunistic pathogen associated with nosocomial infection (6). In 2007, SGI1-L was detected in a *P. mirabilis* clinical isolate, first confirming SGI1 was present in a genus other than *Salmonella* (7). Some novel SGI1 variants have been recently described in *P. mirabilis* (8–11). Three

studies have reported that SGI1-containing *P. mirabilis* could disseminate through meat food consumption, which poses a threat to public health (8, 9, 12), suggesting that SGI1-containing *P. mirabilis* clinical isolates might partly come from food-producing animals. However, data from animal farms are limited.

In the present study, 64 *P. mirabilis* strains isolated from the intestinal tracts of chickens among 11 poultry farms in China between March and December 2012 were screened for the presence of SGI1 by PCR with primers chosen in the left and right junction regions between the chromosome and SGI1 (Table S1 in the sup-

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TABLE 1 SGI1-containing *P. mirabilis* isolates characterized in this study

Strain	Province of isolation	Date of isolation	Antibiotic resistance profile ^a	Resistance genes in MDR region	SGI1 variants	
					Type	Size (kb)
Pm13	Shandong	5 March 2012	AMP, CHL, FFC, NAL, NOR, STR, SPT, DOX, TMP, SUL, SXT	<i>aadA2, floRc, tet(G), bla_{PSE-1}, sul1</i>	SGI1	42.4
Pm107	Anhui	19 March 2012	AMP, CHL, FFC, NAL, STR, SPT, DOX, TMP, SUL, SXT	<i>aadA2, floRc, tet(G), dfrA1, sul1</i>	SGI1-I	42.5
PmC105	Tianjin	21 March 2012	AMP, AMC, NAL, NOR, CIP, STR, SPT, DOX, TMP, SUL, SXT	<i>aadA2, lnuF, sul1</i>	SGI1-W	33.9
PmC162	Hebei	17 October 2012	NAL, NOR, CIP, STR, SPT, DOX, TMP, SUL, SXT	<i>dfrA25, qnrB2, sul1</i>	SGI1-X	38.4
PmX59	Liaoning	1 November 2012	AMP, CHL, NAL, STR, SPT, DOX, TMP, SUL, SXT	<i>dfrA1, sul1</i>	SGI1-O	33.2
PmX60	Sichuan	28 November 2012	AMP, AMC, CTX, CHL, FFC, NAL, STR, SPT, GEN, DOX, TMP, SUL, SXT	<i>aacCA5, aadA7, sul1</i>	SGI1-Y	33.6

^a AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; CHL, chloramphenicol; FFC, florfenicol; NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; STR, streptomycin; SPT, spectinomycin; GEN, gentamicin; DOX, doxycycline; TMP, trimethoprim; SUL, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole. Resistance to antibiotics conferred by SGI1 is indicated in bold type.

FIG 1 Schematic view of three novel SGI1 variants, SGI1-W, SGI1-X, and SGI1-Y. Genes and ORFs are shown as arrows, and their orientations of transcription are indicated by the arrowheads. DR-L and DR-R represent the 18-bp direct repeats at the ends of SGI1. CS, conserved segment; IRI and IRt, inverted repeats defining the left and right ends of class 1 integron, respectively; orf, open reading frame; CR1, common region 1.

plemental material) (2, 8, 9). The left and right junctions of SGI1 were detected in six isolates. They displayed various resistance profiles, as determined by the disk diffusion method according to the CLSI guideline (13). All six SGI1-containing strains harbored different gene cassettes and were not clonally related, as determined by pulsed-field gel electrophoresis. The origins and antibiotic resistance profiles of these strains are listed in Table 1.

In the six strains, the SGI1 integrated into the specific attachment site *attB*, corresponding to the last 18 bp at the 3'-end of the chromosomal *trmE* gene (formerly named *thdF*) by site-specific recombination between the *attB* and the SGI1 *attP* sites (3). The complete SGI1 structures (backbone and MDR region) were established by PCR linkage and sequencing using primers listed in Table S1 in the supplemental material (11). Through PCR mapping, the complete nucleotide sequences of the six SGI1s ranged from 33.2 to 42.5 kb. The SGI1 backbones differed by only several single-base changes from that of *S. Typhimurium* DT104 (2). The

MDR regions were integrated by the class 1 integron belonging to the In4 family and inserted between the backbone genes *res* (S027) and *S044*. Three SGI1s (SGI1, SGI1-I, and SGI1-O) have been previously reported (2, 4, 8). Three new variants, SGI1-W, SGI1-X, and SGI1-Y, have been characterized in this study for the first time (Fig. 1).

InSGI1-W (6.54 kb) contained only the *aadA2/lnuF* cassette, conferring resistance to spectinomycin, streptomycin, and lincosamides. The *aadA2/lnuF* cassette observed in SGI1-W was found in just two species of *Enterobacteriaceae*, *Escherichia coli* (GenBank accession number JF806502) and *Salmonella enterica* (14). This new cassette in SGI1 might be caused by the homologous recombination of integrons among different species in the intestinal microecological environment. The wide use of lincosamides in animal farms might facilitate its occurrence.

InSGI1-X (11.03 kb) contained the *dfrA25* cassette, common region 1 (CR1), and fluoroquinolone resistance gene *qnrB2*. The

major part of this structure harboring *qnrB2* differed only by 3 single-base changes from the 5.24 kb of the partial plasmid p137.25 and 7 single-base changes from the 7.45 kb of the partial plasmid pSE936/05 in *S. enterica* (15, 16), suggesting that the newly found MDR region in SGI1-X might derive from plasmids through homologous recombination of integrons. It is worth noting that SGI1-V recently found in *P. mirabilis* also contained two new antibiotic resistance genes (*bla*_{VEB-6} and *qnrA1*), conferring resistance to the third-generation cephalosporins and fluoroquinolones (10), revealing that SGI1 could improve bacterial adaptability to environmental stress through ongoing acquisition of antibiotic resistance genes.

The SGI1 in the *PmX60* strain was located between the chromosomal *trmE* gene and the gene encoding a membrane protein PMI3124 due to the loss of the *hipBA* module that appeared to occur by homologous recombination between 49-bp direct repeats surrounding the module (17). It contained the *aacCA5-aadA7* cassette that was identical to SGI1-*PmABB* and SGI1-*PmMAT* (11), but the backbone did not include a deletion from S005 to S009 [*aacCA5* has also been called *aac(3)-Id*]. Therefore, this SGI1 was considered a new variant named SGI1-Y. It might derive from the SGI1-B prototype via the cassette replacement.

In conclusion, this study highlights that poultry farms are an important reservoir of SGI1-containing *P. mirabilis*. Three novel SGI1 variants were identified in this study for the first time. In addition, it has been demonstrated that some virulence-enhancing properties in *Salmonella* were associated with the presence of SGI1 (18, 19). Notably, four of the six isolates in this study caused severe peritonitis. The prevalence of SGI1 in animal-origin *P. mirabilis* poses a threat to public health, as SGI1-producing *P. mirabilis* from poultry farms could spread to humans through food consumption, and SGI1 in *P. mirabilis* could spread to *Salmonella* (12, 20).

Nucleotide sequence accession numbers. The complete nucleotide sequences of six SGI1s observed in this study were submitted to GenBank and assigned accession numbers KJ186153 (SGI1 in *Pm13*), KJ186152 (SGI1-I in *Pm107*), KJ186151 (SGI1-W in *PmC105*), KJ186154 (SGI1-X in *PmC162*), KJ186150 (SGI1-O in *PmX59*), and KJ186149 (SGI1-Y in *PmX60*).

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