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Research paper

Distribution and characteristics of SGI1/PGI2 genomic island from *Proteus* strains in China

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ABSTRACT

The emergence of multidrug-resistant *Salmonella* genomic island 1 (SGI1) and *Proteus* genomic island (PGI) bearing *P. mirabilis* present a serious threat to public health. In this study, we screened 288 *Proteus* isolates recovered from seven provinces in China. Fourteen strains (4.9%) all belonged to *P. mirabilis* were positive for SGI1/PGI2, including twelve from clinical samples (5.3%) and two from food (3.3%). A Blastn search against GenBank and phylogenetic analyses identified eight different SGI1 variants and one PGI2 variant from the fourteen SGI1/PGI2 variants. All SGI1 variants shared a common backbone and harbored different resistance gene(s), except the *sul1* gene at its multidrug-resistant (MDR) region. Among the variants, three novel SGI1 variants, designated as SGI1-PmCA11, SGI1-PmCA14 and SGI1-PmCA46, contained different gene cassettes, which were similar to sequences in plasmids or class 1 integrons of *Klebsiella pneumoniae*, *P. mirabilis*, *Escherichia coli* and *Salmonella*. Moreover, one novel PGI2, designated as PGI2-PmCA72, had an identical gene cassette to the first class 1 integron from PGI2 (GenBank accession no. MG201402.1) in *P. mirabilis*, but varied due to missing, replaced, inserted and inverted gene clusters. The four novel SGI1/PGI2 variants contained the *cmlA5*, *dfrA14*, bla_{OXA-10}, *aadA15*, bla_{OXA-1}, *catB3* and *dfrA16* resistance genes, which have never been reported in SGI1/PGI2 variants. Phenotypically, all fourteen SGI1/PGI2-containing strains showed multidrug resistance. All except four strains were resistant to the first, or the second and/or-third generation cephalosporins. Considering the increasing number and the emergence of new SGI1/PGI2 variants, further surveillance is needed to prevent the spreading of the MDR genomic islands among *Proteus* isolates from human and food.

1. Introduction

A genomic island (GI) is part of a genome that is acquired through horizontal gene transfer (Dobrindt et al., 2004; Juhas et al., 2009), and GIs contain many genes that are involved in integration/excision, conjugative transfer, regulation, antibiotic resistance and fitness. *Salmonella* genomic island 1(SGI1) is a 42.4-kb DNA integrative mobilizable genetic element, which was initially found in *Salmonella enterica* serovar Typhimurium DT104 (ST DT104) (Boyd et al., 2001; Doublet et al., 2005) and then in other serovars (Beutlich et al., 2011; Boyd et al., 2002; Doublet et al., 2003; Ebner et al., 2004; Levings et al., 2005). SGI1 consists of a backbone region and a multidrug-resistant (MDR) region, which is a complex class 1 integron named In104 (Hall, 2010). Many isolates of serovar Typhimurium DT104 contain antibiotic-resistance genes that confer resistant to the following a core group of antimicrobials: ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamides (Su), and tetracycline (T) (abbreviated as ACSSuT).

Since the identification of SGI1 in ST DT104 strains, more than 50 different SGI1 variants have been described, due to insertion/deletion of sequence in the backbone region, homologous recombination and/or transposition of gene cassettes at the MDR region. The SGI1 variant in *Proteus mirabilis* was first reported in 2006 from a clinical strain isolated from a patient with diabetic foot infection (Ahmed et al., 2006). SGI1 variants from *P. mirabilis* have been reported in China, France, Africa, Korea and Europe from patients (clinical) and/or animals (Bi et al.,

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2011; Doublet et al., 2010; Schultz et al., 2015; Siebor and Neuwirth, 2013; Soliman et al., 2018a; Sung et al., 2017), and only two variants have been reported from food (Bi et al., 2011; Boyd et al., 2008). SGI1 have also been recently reported from *Morganella morgani* (Schultz et al., 2017a) and *Providencia stuartii* (Soliman et al., 2018b). Furthermore, *Proteus* genomic island 1 (PGI1), *Proteus* genomic island 2 (PGI2) and GIPmi1 inserted into the same location as SGI1 have also been reported. Compared with SGI1, however, the S023–S024 region is missing from PGI1 (Siebor and Neuwirth, 2014), PGI2 is missing the res gene and the S023-S026 region in SGI1 but has an emerging region of PGI2_067–068 (Lei et al., 2018b). The *P. mirabilis* PmPHI strain also contains an integrative and conjugative elements (ICEs) named ICE*Pmi*Fra1 (Siebor et al., 2018).

Proteus is opportunistic pathogens, especially *P. mirabilis*, commonly responsible for urinary tract infections (UTIs), particularly in patients undergoing long-term catheterization (Schaffer and Pearson, 2015). Recently, several SGI1/PGI1 variants containing various resistance genes have been detected. These resistance genes include extended-spectrum β-lactamase-encoding (ESBL) gene and *qnr* in SGI1-V (Siebor and Neuwirth, 2011). The *mphR-mrx-mphA* gene cluster coding resistance to macrolides is found in SGI1-B2 (Lei et al., 2015); SGI1-Z with conjugative NDM-1 plasmid has been detected in a urine sample (Qin et al., 2015). Other resistance genes include SGI1-K7 harboring $bla_{CTX\text{-}M\text{-}15}$ (de Curraize et al., 2018); ESBL $bla_{VEB\text{-}6}$ and carbapenemase-encoding $bla_{NDM\text{-}1}$ genes in PGI1-PmPEL (Girlich et al., 2015), as well as ESBL gene ($bla_{VEB\text{-}6}$) with AmpC gene ($bla_{CMY\text{-}2}$) or $bla_{DHA\text{-}16}$ genes. The presence of PGI2 results in similar typical resistance phenotype, ACSSuT, with fourteen resistance genes, except for the tetracycline resistance gene *tet(J)* (Lei et al., 2018b). Moreover, SGI1/PGI1/PGI2/GIPmi1 can be transmitted by the IncA/C conjugative helper plasmid at high frequency (Douard et al., 2010).

An increasing number of MDR *P. mirabilis* strains isolated from clinical patients, animals and food have become a great threat to public health. However, the prevalence of SGI1/PGI2-bearing *P. mirabilis* strains in clinical and food specimens in China has not been assessed, and only a few studies have identified scattered SGI1-bearing *P. mirabilis* from clinical isolates (Bi et al., 2011; Boyd et al., 2008). The present study aimed to reveal the distribution and molecular characteristics of SGI1/PGI2 in *Proteus* strains from clinical and food isolates in China. The SGI1/PGI2-specific gene was screened from 288 *Proteus* strains isolated from clinical samples and food in China from 2008 to 2015. The genetic structure and evolutionary origins of SGI1 and PGI2 were analyzed in the fourteen *P. mirabilis* strains that were positive for the SGI1/PGI2-specific gene. Furthermore, the role of SGI1/PGI2 in drug resistance was characterized.

2. Materials and methods

2.1. Bacterial strains

In the present study, 288 *Proteus* strains were collected between 2008 and 2015 in China, and they were isolated from clinical (n = 228) and food (n = 60) specimens, distributed in seven provinces, i.e., Beijing, Tianjin, Anhui, Fujian, Jiangsu, Yunnan and Shandong. All strains were grown at 37 °C on Luria-Bertani (LB) plates and identified as *Proteus* through a biochemical test (API 20E, BioMerieux, Lyon, France), including 210 strains of *P. mirabilis* (187 clinical isolates and 23 food isolates), 61 strains of *P. vulgaris*, 15 strains of *P. penneri* and 2 strains of *P. hauseri*. All isolates were stored at −80 °C in LB broth containing 20% glycerol prior to use.

2.2. PCR screening and genome sequencing

All strains were screened for the presence of SGI1/PGI2 using a PCR-based method targeting for all known SGI1 and PGI2 variant integrase gene ($int_{SGI1/PGI2}$) with the previously described 5'CS and 3'CS primers (Schultz et al., 2017b) (Supplementary Table S1). The $int_{SGI1/PGI2}$ positive isolates were then analyzed for the gene cassettes of class 1 integron(s) and the left and right junction regions using previously reported primers (Boyd et al., 2008) (Supplementary Table S1). Next-generation sequencing (NGS) was performed for the positive strains. Genomic DNA was extracted from 2 mL of overnight cultures with a Wizard® Genomic DNA Purification kit (Promega, USA) according to the technical manual. The extracted DNA was stored at −80 °C prior to sequencing. Whole-genome sequencing was performed on the Illumina HiSeq PE150 platform. For library construction, adenine nucleotides were added to the tails and ligated to paired-end adaptors followed by PCR amplification using a 350 bp insert at the Beijing Novo gene Bioinformatics Technology Co., Ltd. After filtering the low quality reads, all good quality paired reads were assembled using SOAP de novo (version 2.04).

2.3. Extraction, assembly and annotation of SGI1/PGI2 variants

The contigs of the variants of thirteen SGI1 and one PGI2 were extracted and assembled from the whole sequenced genomes using SOAP de novo v2.04 (Li et al., 2008; Li et al., 2010) against the reference SGI1 in the genome of ST DT104 (GenBank accession no. AF261825.2) and PGI2 in the genome of the *P. mirabilis* strain BC11–24 (GenBank accession no. MG201402.1). The relationships between contigs were shown by ContigScape (Tang et al., 2013) with custom primer walking. Sanger sequencing was used to close the gaps, and the results were confirmed by PCR. Putative functions were inferred using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast) and the ORF finder (http://www.ncbi.nlm.nih.gov/projects/gorf). The Rapid Annotation using Subsystem Technology (RAST, version 2.0) server pipeline was used to predict open reading frames (ORFs) and annotate the ORFs of the new SGI1/PGI2 variants.

2.4. Cluster analysis of SGI1/PGI2 variants

Each of the SGI1/PGI2 variants was used in a BLAST search to obtain all high homology SGI1/PGI2 variants in the public database (https://www.ncbi.nlm.nih.gov/) (last updated in Jun 2018). Twelve SGI1/PGI2 variants were scanned and selected as references representative of the different types based on different scores and identifications (Supplementary Table S2). To construct the cluster of strains, a non-redundant homologous gene set (234) of all SGI1/PGI2 variants was identified using cd-hit software, and blast 2.2.31 + was then used to verify whether each gene existed in the non-redundant homologous gene set. A matrix with 26 (the number of strains)*234 was constructed. If the gene existed in the strain, the matrix item was denoted as 1, and if the gene did not exist in the strain, the matrix item was denoted as 0. Finally, a cluster of strains was built based on the matrix using R 3.4.4.

2.5. Antibiotic susceptibility testing

The antimicrobial susceptibility phenotypes were determined with a broth dilution method according to the standards and interpretive criteria described by the Clinical and Laboratory Standards Institute (CLSI), CLSI M100-S27 (2017).The following thirty antibiotics were used: ampicillin (AMP), 64 μg; ampicillin/sulbactam (AMS), 64/32 μg; tetracycline (TET), 32 μg; chloramphenicol (CHL), 64 μg; trimethoprim/sulfamethoxazole (SXT), 8/152 μg; cefazolin (CFZ), 16 μg; cefotaxime (CTX), 8 μg; cefotaxime/clavulanic acid (CTX−C), 4/4 μg; ceftazidime (CAZ), 16 μg; ceftazidime/clavulanic acid (CAZ−C), 8/4 μg; cefoxitin (CFX), 64 μg; gentamicin (GEN), 32 μg; imipenem (IMI), 8 μg; nalidixic acid (NAL), 64 μg; azithromycin (AZI), 64 μg; sulfisoxazole (SUL), 512 μg; ciprofloxacin (CIP), 32 μg; amoxicillin/clavulanic acid (AMC), 64/32 μg; colistin (CT), 16 μg; polymyxin B (PB), 16 μg; minocycline (MIN), 32 μg; amikacin (AMI), 128 μg; aztreonam (AZM), 32 μg; cefepime (FEP), 16 μg; meropenem (MEM), 4 μg; levofloxacin

(LEV), 8 μg; doxycycline (DOX), 16 μg; kanamycin (KAN), 64 μg; streptomycin (STR), 32 μg; and gemifloxacin (GEM),16 μg. The plates were custom-made from Shanghai Biofosun Co. Ltd. (Shanghai, China), and the results were recorded based on the color or turbidity according to the company's instructions. The *Escherichia coli* ATCC 25922 reference strain was included as a quality control.

2.6. Nucleotide sequence accession numbers

The sequences and annotated genes of the fourteen SGI1/PGI2 variants in *Proteus* species were submitted to GenBank under accession number MH990670-MH990683.

3. Results

3.1. Distribution and general features of SGI1/PGI2 variants

Fourteen out of 288 (4.9%) *Proteus* isolates belonged to *P. mirabilis* (6.7%, 14/210) and were positive for the $int_{SGI1/PGI2}$ gene, including clinical (n = 12, 5.3%) and food (n = 2, 3.3%) isolates. Eight different SGI1 variants and one PGI2 variant were identified from the fourteen strains (Table 1). Among them, ten SGI1 variants were identified as the following five known SGI1 variants: SGI1-B, SGI1-C, SGI1-I, SGI1-O and SGI1-Z. However, the SGI1-PmCA11, SGI1-PmCA14, SGI1-PmCA46 and PGI2-PmCA72 host strains were all isolated from clinical with no identity sequence of known SGI1/PGI2 variants, indicating that these variants were characterized for the first time. The general genomic features of the SGI1/PGI2 variants are listed in Table 1. The length of the SGI1 variants ranged from 33,832 to 43,577 bp with an average length of 36,873 bp. The G + C content ranged from 45.3% to 50.0%, with a mean of 46.3%. The number of predicted CDSs was between 39 and 53. The length of PGI2-PmCA72 was 46,831 bp, and the G + C content of PGI2-PmCA72 was 48.6% with 40 predicted CDSs.

3.2. Blastn search of closely related SGI1/PGI2 variants in GenBank

A GenBank Blastn search based on several indicators, such as query coverage, identity, max score and total score, showed that thirteen SGI1 variants and one PGI2 variant were closely related to many different GenBank SGI1/PGI2 variants derived from *P. mirabilis* and *Salmonella enterica* (Supplementary Table S3). According to the closely related scores among the hits of each SGI1/PGI2 variant, the top three alignment results are listed for each SGI1/PGI2 variant from different sources. The query cover ranged from 77% to 100% with the similar identity of 99%. The total scores ranged from 6.22E + 04 to 8.60E + 04, and the max score was between 3.31E + 04 and 7.88E + 04. Five known SGI1 variants had the highest query cover of 100% and identity of 99%, while three novel SGI1 variants had a query cover ranging from 77% to 95% and identity of 99%. Nevertheless, PGI2-PmCA72 was closely related to PGI2 detected in the BC11-24 *P. mirabilis* strain with query cover and identity of 91% and 99%, respectively.

3.3. Phylogenetic analysis of SGI1/PGI2 in Proteus isolates

Based on the homologous gene phylogenetic analysis, the fourteen GIs were grouped into two groups as follows: SGI1 and PGI2 (Fig. 1). Group SGI1 was further divided into eight clusters. Cluster I contained four identical SGI1 variants from four *P. mirabilis* strains isolated from clinical samples that were closely related to the SGI1-PmBRI reference (also named SGI1-C) of the PmBRI *P. mirabilis* strain (from a stool sample in France) (Supplementary Table S2). Two identical SGI1 variants (strains isolated from clinical samples) belonged to cluster II with the SGI1-B reference, which was contained in the clinical SLB1 *P. mirabilis* strain. Two identical SGI1 variants (one host strain isolated from food and another from clinical) belonged to cluster III with the SGI1-O reference from the SLO1clinical *P. mirabilis* strain in China. Cluster IV and V each contained only one SGI1 variant with the SGI1-I reference (detected in the Pm107 *P. mirabilis* strain isolated from chicken in China, in 2012) and the SGI1-Z reference (in the PM58 *P. mirabilis* strain isolated from a urine sample in China), respectively. The remaining three SGI1 variants were grouped into three distance clusters (VI, VII and VIII) with no reference. Thus, these three SGI1 variants were designated as novel SGI1 variants.

The PGI2 group included only one cluster detected in the CA150272 strain isolated from a clinical sample with the PGI2 reference from *P. mirabilis* isolated from swine in China (Supplementary Table S2).

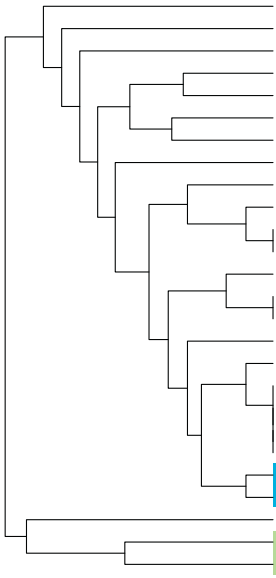
3.4. Characterization of the SGI1/PGI2 variants

All SGI1 variants were inserted into the specific *attB* attachment site at the 3′-end (last 18 bp) of the chromosomal *trmE* gene (formerly known as *thdF*) with one and/or two class 1 integron(s) at the MDR region, and they shared a common backbone that was identical to most SGI1 variants (Table 2, Fig. 2). All the SGI1 variants shared the same sequence (5′-TTCTGTATTGGGAAGTAA-3′) of *attB* and *attP* except SGI1-C which with a C not T at position nine. The sequence of *attB* and *attP* in PGI2-PmCA72 was identity to PGI2. Surprisingly, there is A at position seventeen of *attP* of SGI1 but G in PGI2. All SGI1/PGI2 variants harbored different resistance gene(s), except the *sul1* gene, at their MDR region. Interestingly, the *dfrA1-orfC* gene cassette in the CA120914 strain and the *dfrA17-aadA5* gene cassette in the CA140746 and CA151922 strains were not located at the MDR region.

The three novel SGI1 variants contained different gene cassettes

Table 1
Characterization of SGI1/PGI2 in this study.

Strain ID	Year of isolation	Origin of isolation	SGI1/PGI2	SGI1/PGI2		No. of predicted CDSs (RAST)
				Size (bp)	G + C (%)	
CA140746	2014	Clinical	SGI1-B	35,499	45.3	41
CA151922	2015	Clinical	SGI1-B	35,499	45.3	41
CA141651	2014	Clinical	SGI1-C	35,995	45.6	40
CA150323	2015	Clinical	SGI1-C	35,995	45.5	40
CA150801	2015	Clinical	SGI1-C	35,995	45.6	40
CA152390	2015	Clinical	SGI1-C	35,995	45.6	40
09MAS2416	2009	Food	SGI1-I	43,490	48.9	50
08MAS2393	2008	Food	SGI1-O	33,832	45.8	41
CA150076	2015	Clinical	SGI1-O	35,544	45.4	41
CA151313	2015	Clinical	SGI1-Z(KP662516.1)	34,747	46.4	41
CA121511	2012	Clinical	SGI1-PmCA11	35,236	46.2	39
CA120914	2012	Clinical	SGI1-PmCA14	43,577	50.0	53
CA140446	2014	Clinical	SGI1-PmCA46	37,957	46.2	46
CA150272	2015	Clinical	PGI2-PmCA72	46,831	48.6	40



Cluster with the homologous genes	Strain name	GI variants	Isolation year	Isolation place	Isolation source	SGI1/PGI2 cluster
	Proteus mirabilis isolate Pm37THOMI	SGI1-Pm37THOMI (SGI1-K7)	2013	France	urine	
	Proteus mirabilis strain VB1248	SGI1-V	2009	France	Blood culture	
	Proteus mirabilis isolate Pm2CHAMA	SGI1-Pm2CHAMA	2013	France	urine	
	Proteus mirabilis 09MAS2416*	SGI1-I	2009	China	food	SGI1_cluster IV
	Proteus mirabilis strain Pm107	SGI1-I	2012	China	chicken	
	Morganella morganii strain Pr5	-	2016	France	clinical	
	Salmonella enterica subsp. *enterica* serovar erro strain SRC5	SGI1-F	1999	Australia	traveler	
	Proteus mirabilis CA120914*	SGI1-PmCA14	2012	China	clinical	SGI1_cluster VI
	Proteus mirabilis CA140446*	SGI1-PmCA46	2014	China	clinical	SGI1_cluster VII
	Proteus mirabilis strain SLB1	SGI1-B	2012	China	patient	SGI1_cluster II
	Proteus mirabilis CA151922*	SGI1-B	2015	China	clinical	
	Proteus mirabilis CA140746*	SGI1-B	2014	China	clinical	
	Proteus mirabilis 08MAS2393*	SGI1-O	2008	China	food	
	Proteus mirabilis CA150076*	SGI1-O	2015	China	clinical	SGI1_cluster III
	Proteus mirabilis strain SLO1	SGI1-O	2013	China	patient	
	Proteus mirabilis CA121511*	SGI1-PmCA11	2012	China	clinical	SGI1_cluster VIII
	Proteus mirabilis CA150801*	SGI1-C	2015	China	clinical	
	Proteus mirabilis strain PmBRI	SGI1-PmBRI(SGI1-C)	2011	France	stool	
	Proteus mirabilis CA150323*	SGI1-C	2015	China	clinical	SGI1_cluster I
	Proteus mirabilis CA152390*	SGI1-C	2015	China	clinical	
	Proteus mirabilis CA141651*	SGI1-C	2014	China	clinical	
	Proteus mirabilis CA151313*	SGI1-Z	2015	China	clinical	SGI1_cluster V
	Proteus mirabilis strain PM58	SGI1-Z(KP662516.1)	2013	China	urine	
	Acinetobacter baumannii strain D4	AGI1	2006	Australia	wound	
	Proteus mirabilis CA150272*	PGI2-PmCA72	2015	China	clinical	PGI2_cluster
	Proteus mirabilis strain BC11-24	PGI2	2016	China	swine	

Fig. 1. The cluster of strains based on the homologous genes of the fourteen SGI1/PGI2 variants (asterisk).

that have not been reported in SGI1-positive *P. mirabilis* to date except for SGI1-PmCA11, and mercury resistance genes were detected in SGI1-PmCA14 (Fig. 2). A GenBank Blastn search indicated that the sequences of three novel gene cassettes were closely related to many different class 1 integron or plasmid sequences (Table 3). Only the *aadB-orf416-cmlA5* gene cassette detected in SGI1-PmCA14 appeared integral on plasmids or class I integron with a query cover and identity of 99% and 100%, respectively. The other two gene cassettes were not adjacent on the detected plasmids. The *dfrA14-arr-2-bla*$_{OXA-10}$*-aadA15* gene cassette in SGI1-PmCA11 was similar to sequences of *Klebsiella pneumonia*, *P. mirabilis* and *Escherichia coli* plasmids with a query cover and identity of 100% and 99%, respectively. In addition, the *pse-1-aacA4-bla*$_{OXA-1}$*-catB3-arr-3-dfrA17-aadA5* gene cassette in SGI1-PmCA46 was identical to a partial (81%) sequence in *Escherichia coli* and *Salmonella* plasmids. The MDR region of PGI2-PmCA72 included *dfrA16*, *pse-1*, *aadA2*, *cmlA1*, *aadA1*, *sul1*, *floR*, *tetA* and *tetR* resistance genes (Table 2, Fig. 2). Compared to the PGI2 reference (accession no. MG201402.1) detected in the BC11-24 *P. mirabilis* strain, the *dfrA16-pse-1-aadA2-cmlA1-aadA1* gene cassette was identical to the first class 1 integron, and the region from PGI2_034 to *glmM* was missing from PGI2-PmCA72. Further, the *tetA-tetR-relaxase-tnpA* (approximately 3.7 kb) gene cluster replaced the region from PGI2_053 to *sul1_2*, and *hipB/A* genes were inserted between PGI2_068 and PMI3124 with an inverted *rcr2* gene and *lysR-floR*-PGI2_052 gene cluster.

3.5. MDR phenotypes of the fourteen strains and the relationship with SGI1/PGI2

Phenotypically, all fourteen SGI1/PGI2-positive strains showed multidrug resistance (Table 2). The CA120914 and CA151313 isolates (most antibiotic phenotypes) were resistance to twenty drugs, while the least three resistance strains, namely, CA121511, CA150323 and CA150801, were resistance to ten drugs. Notably, all isolates were resistant to TET, MIN, DOX, SUL, STR, GEM, CT and PB, except the CA121511 strain, which was sensitive to GEM, and the CA140446 strain, which showed intermediate resistance to STR. These SGI1/PGI2 variants contained the following genes: *aadA1*, *aadA2*, *aadA15*, *aadB* and *aacA4*, which encode resistance to aminoglycosides; *dfrA1*, *dfrA12*, *dfrA14*, *dfrA16* and *dfrA17*, which confer resistance to trimethoprim; *pse-1/bla*$_{PSE-1}$, *bla*$_{OXA-1}$ and *bla*$_{OXA-10}$, which confer resistance to β-lactams; and *cmlA1* and *cmlA5*, which encode resistance to chloramphenicol. Surprisingly, the CA140446 strain was not resistance to chloramphenical and aminoglycosides though it contained *catB3* and *aadA5* genes (Table 2). All isolates, except CA121511, CA150801, CA150076 and CA150323, were resistance to the first (CFZ), or the second (CFX) and/or-third (CTX, CAZ, CTX-C and CAZ−C) generation cephalosporins. The CA120914 strain was even resistance to the fourth generation cephalosporin (FEP). Except for the CA150272 strain, the phenotypes of the other strains had partial of ACSSuT.

4. Discussion

In the present study, the total isolation rate of SGI1/PGI2-bearing *Proteus* was 4.9%, and the SGI1/PGI2 variants were contained only in *P. mirabilis* strains (6.7%). The clinical and food isolation rates of SGI1/PGI2-bearing *P. mirabilis* were 6.4% and 8.7%, respectively. The total isolation rate was less than that for SGI1 and/or PGI1-bearing *P. mirabilis* strains from clinical samples in France (Schultz et al., 2015; Siebor and Neuwirth, 2013). There are only two reports on SGI1/PGI2-bearing *P. mirabilis* in food (Bi et al., 2011; Boyd et al., 2008). Diverse variants of SGI1/PGI1 have been reported in both *P. mirabilis* and *Salmonella*, such as SGI1-O (Boyd et al., 2008; Siebor and Neuwirth, 2013) and SGI1-I (Boyd et al., 2008; Lei et al., 2014), while other variants have been reported in *P. mirabilis* (Lei et al., 2014; Girlich et al., 2015; Lei et al., 2015; Qin et al., 2015; Siebor and Neuwirth, 2014; Soliman et al., 2017). However, fourteen strains in the present study contained eight SGI1 variants (five known and three novel ones) and one PGI2 variant. Moreover, PGI2 was only detected in *P. mirabilis* in China, and the backbone of PGI2 was related to SGI1 with partial backbone of PGI2 (98% identical). The amino acid sequence of Int$_{PGI2}$ had 97% and 89% identities with those of Int$_{PGI1}$ and Int$_{SGI1}$, respectively, and the PGI2_066 ORF was 97% identical to that of S044 of SGI1 (Lei et al., 2018b). Taken together, these results indicated high diversity of MDR in SGI1/PGI2 in Chinese *P. mirabilis* strains. Multiple horizontal transfer events of SGI1/PGI1/PGI2 elements occurred in *P. mirabilis*, as previously observed in different *S. enterica* serovars (Djordjevic et al., 2009; Le Hello et al., 2012; Targant et al., 2010). As a result, more GI-bearing *P. mirabilis* harboring diverse antimicrobial resistance genes have been reported, and reports about *P. mirabilis* strains carrying GI variants play a role as an opportunistic MDR pathogen to human and

Table 2
Characterization and antibiotic resistance patterns of SGI1/PGI2 contained strains in this study.

Strain ID	SGI1/PGI2	Integron cassette(s)[a]		Antibiogram[b]	Other resistance genes
		Gene (s)	Size (s) (kb)		
CA140746	SGI1-B	*pse-1*, ***dfrA17-aadA5***	1.6, 1.2	**AMP**, CFZ, GEN, STR, TET, MIN, DOX, **SXT**, **SUL**, NAL, LEV, CIP, GEM, CT, PB	*sul1*
CA151922	SGI1-B	*pse-1*, ***dfrA17-aadA5***	1.2, 1.6	**AMP**, AMS, AMC, CFZ, CTX, CTX/C, GEN, STR, TET, MIN, DOX, CHL, **SXT**, **SUL**, NAL, CIP, GEM, CT, PB	*sul1*
CA141651	SGI1-C	*aadA2*	1.0	CFZ, **STR**, TET, MIN, DOX, **SUL**, NAL, LEV, CIP, GEM, CT, PB	*sul1*
CA150323	SGI1-C	*aadA2*	1.0	**STR**, TET, MIN, DOX, **SUL**, NAL, CIP, GEM, CT, PB	*sul1*
CA150801	SGI1-C	*aadA2*	1.0	AZM, **STR**, TET, MIN, DOX, **SUL**, NAL, GEM, CT, PB	*sul1*
CA152390	SGI1-C	*aadA2*	1.0	IMI, CFZ, **STR**, TET, MIN, DOX, **SXT**, **SUL**, NAL, GEM, CT, PB	*sul1*
09MAS2416	SGI1-I	*aadA2*, *dfrA1-orfC*	1.0, 1.2	AMP, CFZ, KAN, STR, TET, MIN, DOX, CHL, **SXT**, **SUL**, NAL, LEV, GEM, CT, PB	*tetR*, *tet(G)*, *sul1*
08MAS2393	SGI1-O	*dfrA1-orfC*	1.2	AMP, CFZ, CTX, **STR**, **TET**, MIN, DOX, CHL, **SXT**, **SUL**, NAL, LEV, CIP, GEM, CT, PB	*sul1*
CA150076	SGI1-O	*dfrA1-orfC*	1.2	AMP, KAN, STR, TET, MIN, DOX, CHL, **SXT**, **SUL**, NAL, GEM, CT, PB	*sul1*
CA151313	SGI1-Z (KP662516.1)	*dfrA12-orfF-aadA2*	1.8	AMP, AMC, CFZ, CTX, CTX/C, AZI, GEN, KAN, **STR**, TET, MIN, DOX, CHL, **SXT**, **SUL**, NAL, LEV, CIP, GEM, CT, PB	*sul1*
CA121511	SGI1-PmCA11	*dfrA14-arr-2-bla*$_{OXA-10}$*-aadA15*	3.1	**AMP**, AMS, **STR**, TET, MIN, DOX, **SXT**, **SUL**, CT, PB	*sul1*
CA120914	SGI1-PmCA14	*aadB-orf416-cmlA5*, ***dfrA1-orfC***	1.2, 2.9	AZM, CFX, CFZ, CAZ, CTX/C, CAZ/C, FEP, AZI, GEN, AMI, KAN, **STR**, TET, MIN, DOX, **CHL**, **SXT**, **SUL**, NAL, GEM, CT, PB	*sul1*, *merR/T/P/F/A/D/E*
CA140446	SGI1-PmCA46	*pse-1*, *dfrA17-aadA5*	1.2, 1.6	**AMP**, CFZ, AZI, **TET**, MIN, DOX, SXT,**SUL**, NAL, LEV, CIP, GEM, CT, PB, STR*	*aacA4*, *bla*$_{OXA-1}$, *catB3*, *arr-3*, *sul1*
CA150272	PGI2-PmCA72	*dfrA16-pse-1-aadA2-cmlA1-aadA1*	5.0	**AMP**, CFZ, CTX, AZI, **STR**, **TET**, MIN, DOX, **CHL**, **SXT**, **SUL**, GEM, CT, PB	*sul1*, *floR*, *tetA*, *tetR*

Note: a, gene(s) and size were amplicon produced by primers 5'CS and 3'CS. Bold font genes were not detected at MDR region of SGI1. b, Resistance to the antibiotics shown in bold is conferred by genes present on SGI1/PGI2. asterisk means the strain showed intermediate resistance to the antibiotic. Abbreviations: AMP: ampicillin, AMS: ampicillin/sulbactam, TET: tetracycline, CHL: chloramphenicol, SXT: trimethoprim/sulfamethoxazole, CFZ: cefazolin, CTX: cefotaxime, CTX−C: cefotaxime/clavulanic acid, CAZ: ceftazidime, CAZ−C: ceftazidime/clavulanic acid, CFX: cefoxitin, GEN: gentamicin, NAL: nalidixic acid, AZI: azithromycin, SUL: sulfisoxazole, CIP: ciprofloxacin, AMC: amoxicillin/clavulanic acid, CT: colistin, PB: polymyxin, MIN: minocycline, AMI: amikacin, AZM: aztreonam, FEP: cefepime, LEV: levofloxacin, DOX: doxycycline, KAN: kanamycin, STR: streptomycin, GEM: gemifloxacin.

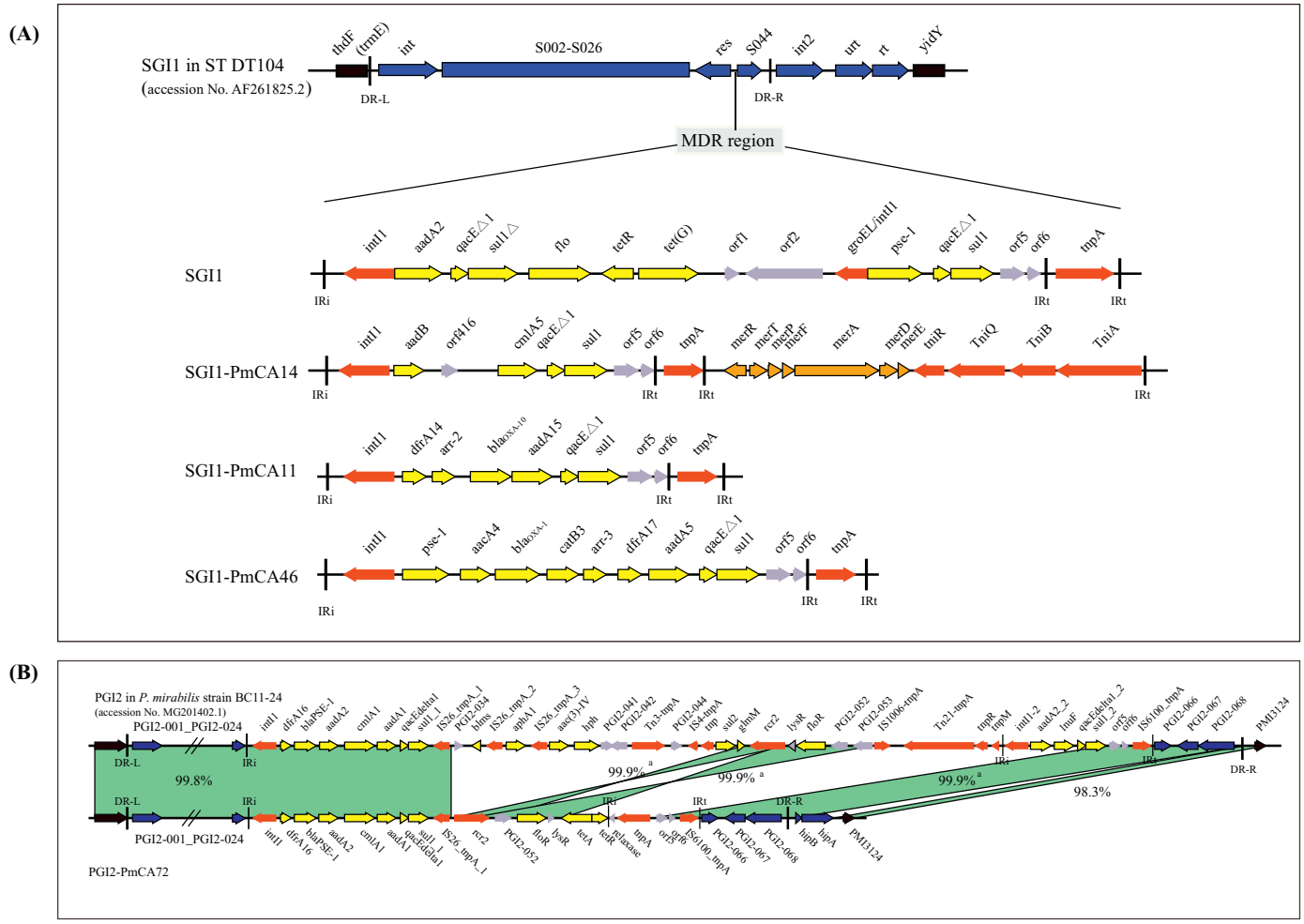


Fig. 2. Genetic organization of the novel SGI1/PGI2 variants in present study. (A) Genetic organization of the three novel SGI1 variants. Arrows indicate ORFs and genes, and their transcriptional orientation. Black rectangles represent chromosomal genes and blue arrows are the backbone. The 18 bp direct repeats (DR-L and DR-R) bracketing the module are shown. IRi and IRt are 25 bp imperfect inverted repeats defining the left and right ends of the MDR region. (B) Comparison of PGI2 (GenBank accession no. MG201402.1) with the novel PGI2-PmCA72. Antibiotic resistance genes are shown as yellow arrows. The orange arrows represent the mercury resistance genes, and transposase or integrase genes are in red. The shaded areas indicate nucleotide identity with the same and inverted orientation. Dash indicated the location of PGI2_002-PGI2_023. [a]Only 1 to 2 nt differences. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
BLASTn search against GenBank of the gene cassettes in novel SGI1 variants.

SGI1 variants	Query genes	Strain	Plasmid/class 1 integron	Query cover (%)	Identity (%)	Accession no.
SGI1-PmCA14	*aadB-orf416-cmlA5*	*Klebsiella pneumoniae*	pKKp4-VIM	99	100	MF582638.1
		Salmonella enterica serovar Heidelberg	pSH696_135	99	100	JN983048.1
		Providencia stuartii	pMR0211	99	100	JN687470.1
		Salmonella sp. s084	class 1 integron	99	100	AB285479.1
		Escherichia coli	class 1 integron	99	100	DQ018383.1
		Salmonella typhimurium	class 1 integron	99	100	AJ487033.2
SGI1-PmCA11	*dfrA14-arr-2-bla*$_{OXA-10}$*-aadA15*	*Klebsiella pneumoniae* strain 160,111	pIncAC2_L111	100	99	CP030132.1
		Proteus mirabilis strain CCUG 70746	pPmi70746_1	100	99	CP023274.1
		Escherichia coli strain AMA566	pAMA566	100	99	MG450360.1
SGI1-PmCA46	*pse-1-aacA4-bla*$_{OXA-1}$*-catB-arr3-dfrA17-aadA5*	*Escherichia coli* strain 974	p974-IncF	81	100	MH580300.1
		Salmonella enterica serovar Indiana strain D90	pD90–1	81	100	CP022451.1

animals (Girlich et al., 2015; Siebor and Neuwirth, 2014; Soliman et al., 2017). In addition to SGI1/PGI1/PGI2, ICEs, such as SXT/R391 (Li et al., 2016) and ICE*Pmi*Fra1 (Siebor et al., 2018), were also found in *P. mirabilis*.

The novel SGI1/PGI2 variants in the present study exhibited several variations of resistant gene cassettes. In SGI1-PmCA14, both *cmlA5* and *cmlA7* encoded resistance to chloramphenical with only 1 bp difference: a T missing at position 2027 in *aadB-orf*416-*cmlA7* to form the *aadB-orf*416-*cmlA5*. The *aadB-orf*416-*cmlA7* gene cluster may have been first transferred into *P. mirabilis* and then a T residue may have been deleted

at 2027, or the T residue may have been deleted at position 2027 followed by a transfer into *P. mirabilis*, thereby forming the novel SGI1-PmCA14. Though SGI1-PmCA46 harbored the *pse-1-aacA4-bla*$_{OXA-1}$-*catB3-arr-3-dfrA17-aadA5* gene cassette, PCR results showed that it contained two gene cassettes, namely, *pse-1* and *dfrA17-aadA5*. Further analysis indicated that the *aacA4-bla*$_{OXA-1}$-*catB3-arr-3* gene cluster has been reported in the ICE*Pmi*Chn-BCP11 (Lei et al., 2018a), ISCR1 PER-4 class 1 integron (Xie et al., 2016), pPm14C18 plasmid (KU605240.1) and GN2 chromosome (CP026581.1) in *P. mirabilis*. The *dfrA17-aadA5* gene cassette has been detected in SGI1-B2 (Lei et al., 2015) in *P. mirabilis*, and the *pse-1* gene has been reported in SGI1-B (Boyd et al., 2002) from ST DT104. Thus, the gene cassette in SGI1-PmCA46 may have been formed through two ways as follows: one way is that SGI1-B first obtained the *aacA4-bla*$_{OXA-1}$-*catB3-arr-3* gene cluster inserted into the end of *pse*-1 and then acquired the *dfrA17-aadA5* genes following the arr-3 gene; and another way is that the *aacA4-bla*$_{OXA-1}$-*catB3-arr-3* gene cluster and *dfrA17-aadA5* gene cluster concatenated first and then transferred into SGI1-B to form the novel SGI1-PmCA46. The structure of PGI2-PmCA72 can be explained by a duplication of the IS*26_tnpA_1* gene located between *glmM* and *rcr2* genes followed by homologous recombination between the two IS*26_tnpA_1* genes, resulting is loss of the region from PGI2_034 to *glmM*. Homologous recombination may also have occurred, resulting in the PGI2_052-*tetA-tetR-relaxase-tnpA-orf5* gene cluster replacing the region from PGI2_052 to *orf5* in PGI2 firstly. An inversion may have also occurred in the *rcr2* gene and *lysR-floR*-PGI2_052 region, and *hipB/A* genes may have been inserted, ultimately generating the PGI2-PmCA72.

To date, the resistance genes detected in SGI1 variants are mainly divided into four types (Boyd et al., 2001; Chu et al., 2012; Doublet et al., 2008; Lei et al., 2015; Siebor and Neuwirth, 2013; Soliman et al., 2017) as follows: (i) aminoglycosides genes: *aadA7*, *aadA5*, *aadA2*, *aadA1*, *aadB*, *aac(3)-Id*, *aacCA5*, *aacA4*,*aac(3)-IIa*, *strB*, *strA*, *aphA1a* and *tmrB*; (ii) β-lactams genes: *bla*$_{PSE-1}$, *bla*$_{TEM-1}$, *bla*$_{TEM-1b}$, *bla*$_{VEB-6}$, *bla*$_{CARB-4}$ and *bla*$_{CTX-M-15}$; (iii) sulfonamides genes: *sul1*, *dfrA1*, *dfrA15*, *dfrA10*, *dfrA5*, *dfrA25*, *dfrA17* and *dfrA12*; and (iv) other resistance genes, including tetracycline genes (*tetR*, *tet(G)* and *tet(A)*, quinolone genes (*qnrA1* and *qnrB2*), macrolides genes (*mphA*, *mphR* and *mrx*), quaternary genes (*qacH* and *qacE*) and a polypeptide gene(*lnuF*). The present study identified three novel SGI1 variants with new resistance genes, namely *cmlA5*, *dfrA14*, *bla*$_{OXA-10}$, *aadA15*, *bla*$_{OXA-1}$ and *catB3*. Surprisingly, the CA140446 strain was sensitive to chloramphenical and intermediate resistance to aminoglycosides, though it contained *catB3* and *aadA5* genes with no mutation. Surprisingly, there is an A inserted into the promoter region of *aadA5*, maybe for the intermediate resistance to aminoglycosides; however, no mutation was found in the promoter region of *catB3*. This phenomenon also appeared in SGI1-M as the strain is sensitive to tetracycline, though *tetR(G)* genes are present in the MDR region (Vo et al., 2007). GIs contain an increasing number of antibiotic resistance genes, and emerging resistance genes detected in *P. mirabilis* isolates may pose a threat to public health, as the isolates can spread to humans through food consumption (Bi et al., 2011; Seiffert et al., 2013), which may prolong hospital stays and increase economy burden for strains isolated from inpatients especially indwelling catheter patients.

P. mirabilis are opportunistic pathogens, which are commonly responsible for UTI, especially for long-term catheterization patients. Nitrofurantoin together with fosfomycin or AMI has better percentages of susceptibility for UTI. In the present study, all 14 GI-positive strains were sensitive to AMI, except the CA120914 strain, and all strains contained fosfomycin resistance gene with 87% identity to *Escherichia coli* mutant glpT, without nfsA or nfsB gene coding resistance to nitrofurantoin. Moreover, eight SGI1-positive strains were isolated from clinical samples. Thus, it is necessary to test drug susceptibility before treating. In conclusion, the distribution and molecular characteristics of SGI1/PGI2 was revealed in *Proteus* strains from clinical and food isolations in China. High diversity and multiple horizontal transfer events of SGI1/PGI2 elements have occurred in *P. mirabilis*. The SGI1/PGI2-bearing strains play a role as opportunistic MDR pathogens to human and animals. Therefore, continuous monitoring of antimicrobial resistance and related mobile elements among *Proteus* isolates from clinical and food is necessary.

Competing interests

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.02.027.

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