



Role of *sul2* Gene Linked to Transposase in Resistance to Trimethoprim/Sulfamethoxazole Among *Stenotrophomonas maltophilia* Isolates

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Dear Editor,

Stenotrophomonas maltophilia has received considerable attention owing to its high infection rate and resistance to multiple antibiotic classes [1]. Trimethoprim/sulfamethoxazole (SXT) is the recommended first-line therapy for *S. maltophilia* infection; however, resistance rate to SXT of *S. maltophilia* have increased worldwide; in Anhui province of China, the resistance rate has increased from 10% to 40% over the past five years [2]. Accordingly, it is important to study the mechanisms underlying SXT resistance and prevent the spread of resistance. We found that *sul1* in combination with *sul2* confers high-level resistance to SXT; *sul1* was always found as part of the 3' end of the class 1 integron, in Anhui province [3]; however, no association between *sul2* and integrons was found.

In the present report, 41 *sul2*-positive *S. maltophilia* samples were collected in one month (September) every year (from 2010 to 2012) from different patients across 31 hospitals in Anhui, China. All *S. maltophilia* isolates were identified using the MicroScan WalkAway 40 System (Dade Behring, Deerfield, IL, USA). *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, and *E. coli* ATCC 25922 were used as quality con-

trol strains for antimicrobial susceptibility testing. Minimal inhibitory concentrations (MICs) of SXT were determined for each isolate by the agar dilution method according to the Clinical and Laboratory Standards Institute [4]. Presence of *sul1*, *sul2*, and *dfrA* in each strain was assessed by PCR as described previously [3].

Of the 41 *sul2*-positive isolates, 30 (73.2%) were resistant to SXT, 28 (68.3%) carried *sul1*, eight (19.5%) carried *dfrA12*, and 10 (24.4%) carried *dfrA17* (Table 1). Twenty-eight of the 30 SXT-resistant and *sul2*-positive isolates contained *sul1* and/or *dfrA*. *Sul1* and *dfrA*, which lead to an increase in the MIC of SXT or SXT resistance in *S. maltophilia*, have been described previously [3].

Sul2 genes linked to insertion sequence common region (ISCR) elements were previously reported [5]. We investigated whether *sul2* could be linked to other mobile elements in this region by thermal asymmetric interlaced PCR (TAIL-PCR), which was developed for amplification of unknown DNA fragments flanked by known sequences. In TAIL-PCR, three nested, sequence-specific primers are utilized in consecutive reactions together with arbitrary degenerate (AD) primers to enhance the

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Table 1. Distribution of the *sul* and *dfrA* genes in 41 *sul2*-positive *Stenotrophomonas maltophilia* isolates

Gene	N of isolates	MIC of SXT (µg/mL)							
		0.25/4.95	1/19	2/38	4/76	8/152	16/304	32/608	>32/608
<i>sul2</i>	13	1	6	4	2				
<i>sul2, sul1</i>	10				2			1	7
<i>sul2, sul1, dfrA12</i>	8						3	1	4
<i>sul2, sul1, dfrA17</i>	10					1	1	3	5

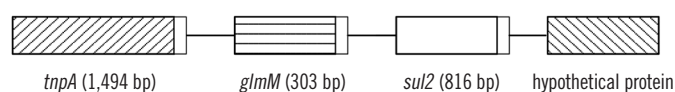
Abbreviations: MIC, minimal inhibitory concentration; SXT, trimethoprim/sulfamethoxazole.

amplification efficiency of specific products [6]. Three specific primers (*sul2*-SP1: AAAGAACGCCGCAATGTGATCC, *sul2*-SP2: GGATAGAACGCAGCGTCTGGAAAA, and *sul2*-SP3: CATCTGCCTTGAGCGCGTCC) and four AD primers (AD1, AD2, AD3, and AD4) from the Genome Walking Kit (TaKaRa Bio, Dalian, China) were used to amplify the 3' flanking sequences of *sul2*. Three specific primers (*sul2*-SP4: GTGGAATGAGTTGGAAGAA, *sul2*-SP5: GTTGCCGTTGTGGGTGCTGT, and *sul2*-SP6: TGCGC-TATCGTGGGGAATG) and the four AD primers were used to amplify the 5' flanking sequences of *sul2*. The specific primers were designed to have a melting temperature (60 to 65°C) that was higher than that of the AD primers (45°C). The flanking sequences of *sul2* were amplified by PCR according to the protocol of the Genome Walking Kit (TaKaRa Bio). PCR products were cloned into the pMD19-T vector and sequenced.

According to results of TAIL-PCR and sequence analysis of the 41 *sul2*-positive isolates, in 27 isolates resistant to SXT, a 2-kb nucleotide sequence encoding transposase (*tnpA2*) and phosphoglucosamine mutase (*glmM*) was obtained for the 5' end of *sul2*; in the same 27 isolates, a 3.2-kb nucleotide sequence encoding a conserved hypothetical protein was obtained for the 3' end of *sul2*. The genetic structure of the flanking nucleotides linked to *sul2* is shown in Fig. 1 and has been deposited in GenBank.

Epidemiological genotyping of the 27 isolates was performed by randomly amplified polymorphic DNA PCR, as described previously [7]. Twenty-three isolates had unrelated genotypes, and the other four isolates were found to share the same genotype. After tracking clinical data, these four isolates were determined to be from the same department of a hospital, indicating that clonal spread was responsible for dissemination of *sul2* among *S. maltophilia* isolates. Presence of *sul2* has increased SXT resistance, when it is associated with transposons; *sul2* could be further disseminated among bacteria through horizontal gene transfer in *S. maltophilia* isolates.

In summary, this is the first report showing the close associa-

**Fig. 1.** Schematic diagram of nucleotides flanking the *sul2* gene.

tion of the SXT resistance gene *sul2* with transposase in *S. maltophilia* isolates. These results suggest that acquired resistance has an important role in the resistance of *S. maltophilia* to SXT, which may increase by means of mobile elements.

Nucleotide sequence accession numbers

Sequences of the *tnpA2-glmM-sul2*-hypothetical protein have been deposited in GenBank and were assigned the accession number JX869967.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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