



The *sul1* Gene in *Stenotrophomonas maltophilia* With High-Level Resistance to Trimethoprim/Sulfamethoxazole

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Emerging resistance to trimethoprim/sulfamethoxazole (SXT) poses a serious threat to the treatment of *Stenotrophomonas maltophilia* infections. We determined the prevalence and molecular characteristics of acquired SXT resistance in recent clinical *S. maltophilia* isolates obtained from Korea. A total of 252 clinical isolates of *S. maltophilia* were collected from 10 university hospitals in Korea between 2009 and 2010. Antimicrobial susceptibility was determined by using the CLSI agar dilution method. The *sul1*, *sul2*, and *sul3* genes, integrons, insertion sequence common region (ISCR) elements, and *dfra* genes were detected using PCR. The presence of the *sul1* gene and integrons was confirmed through sequence analysis. Among the 32 SXT-resistant isolates, *sul1* was detected in 23 isolates (72%), all of which demonstrated high-level resistance (≥ 64 mg/L) to SXT. The *sul1* gene (varying in size and structure) was linked to class 1 integrons in 15 of the 23 isolates (65%) harboring this gene. None of the SXT-susceptible isolates or the SXT-resistant isolates with a minimum inhibitory concentration of 4 and 8 mg/L were positive for *sul1*. Moreover, the *sul2*, *sul3*, and *dfra* genes or the ISCR elements were not detected. The *sul1* gene may play an important role in the high-level SXT resistance observed in *S. maltophilia*.

Key Words: *Stenotrophomonas maltophilia*, Trimethoprim/sulfamethoxazole, *sul*, Class 1 integron

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Stenotrophomonas maltophilia, a non-fermentative, gram-negative bacillus frequently found in community or hospital environments, has emerged as an important opportunistic pathogen; it is most commonly associated with respiratory infections in humans. The incidence of hospital-acquired *S. maltophilia* infections is increasing, and cases of community-acquired *S. maltophilia* have also been reported [1, 2]. The treatment of *S. maltophilia* infections is extremely difficult, owing to the intrinsic or acquired resistance to multiple therapeutic drugs, the development of resistance during therapy, and the paucity of clinical therapeutic data [3]. The recommended first-line agent in the treatment of *S. maltophilia* is trimethoprim/sulfamethoxazole (SXT), be-

cause of low incidence of resistance [1]. However, the treatment of *S. maltophilia* infections has become more problematic, with an increase in the acquired resistance to SXT [4, 5]. The *sul* genes are known to contribute to the resistance to SXT and have been reported to associate with the class 1 integrons and insertion sequence common region (ISCR) elements [6, 7]. Moreover, it has been reported that the *dfra* genes, located in the gene cassettes of the class 1 integrons, lead to a high-level resistance to SXT [8]. However, *sul1* has been detected in the SXT-susceptible *S. maltophilia* isolates [8-10]. In this study, we analyzed the predominant mechanisms underlying acquired SXT resistance in recent clinical *S. maltophilia* isolates obtained from Korea.

A total of 252 non-duplicate clinical isolates of *S. maltophilia* were collected from 10 university hospitals in Korea between 2009 and 2010. The species were identified by using conventional methods and/or the ATB 32GN system (bioMérieux, Marcy l'Etoile, France). The SXT susceptibility was determined by using the CLSI agar dilution method [11]. In order to assess the presence of *sul* genes and integrons in the isolates, PCR was performed with specific primers (*sul1* [Forward, 5'-ATG GTG ACG GTG TTC GGC ATT CTG A-3'; Reverse, 5'-CTA GGC ATG ATC TAA CCC TCG GTC T-3'], *sul2* [Forward, 5'-GAA TAA ATC GCT CAT CAT TTT CGG-3'; Reverse, 5'-CGA ATT CTT GCG GTT TCT TTC AGC-3'], *sul3* [Forward, 5'-CAT TCT AGA AAA CAG TCG TAG TTC G-3'; Reverse, 5'-CAT CTG CAG CTA ACC TAG GGC TTT GGA-3'], and class 1 integron [Forward, 5'-GCC TGT TCG GTT CGT AAG CT-3'; Reverse, 5'-CGG ATG TTG CGA TTA CTT CG-3']) [7, 12]. The presence of *sul1* genes and integrons in the isolates was confirmed by sequencing. PCR analysis was performed by using ISCR-specific primers (ISCR-F, GCG AGT CAA TCG CCC ACT; ISCR-R, CGA CTC TGT GAT GGA TCG AA) to detect ISCR elements in the *sul1*-positive *S. maltophilia* isolates [13], and additional PCR analysis was conducted by using certain primer combinations (e.g., *sul1*-F:ISCR-R or ISCR-F:*sul1*-R). The presence of *dfrA* genes in the isolates was determined as per the method by Seputiene *et al.* [14]. The *dfrA* genes were grouped according to their sequence similarity, and the primers were designed for analyzing the *dfrA* genes. Four groups of primers were used to include all previously reported *dfrA* genes found in *S. maltophilia* [8].

The *sul* genes were detected in 23 SXT-resistant isolates, but not in nine isolates exhibiting low-level SXT resistance (minimum inhibitory concentration [MIC] 4-8 mg/L). Moreover, *sul* was not detected in the 220 SXT-susceptible isolates. All the *sul* genes detected were found to be *sul1* genes; the genes *sul2* and *sul3* were not detected in the isolates. Fifteen of the 23 *sul1*-positive isolates harbored class 1 integrons (Table 1). The integrons varied

in size and structure. Six isolates possessed only *int11* and *sul1*. All the remaining integrons harbored the *qacEΔ1* gene cassettes. One of these integrons also harbored the *qac* gene. Eight isolates contained one or two aminoglycoside-modifying enzyme genes (four isolates: *aac6'-lb*; two isolates: *aac6'-31*-like; one isolate: *aacA7*; one isolate: *aacA7* and *aadA4a*). The PCR amplification products of ISCR were not detected, and the linkage of ISCR to the *sul1* gene was not observed. No *dfrA* genes were detected.

In a previous study, the overall resistance rate of the Korean *S. maltophilia* isolates to SXT was low (4%), while the resistance rate in one hospital was very high (26%) [15]. This result indicates that the empirical selection of SXT for the treatment of *S. maltophilia* infections in some Korean hospitals may be inadequate for curing this infection completely. Additionally, Song *et al.* [10] reported that 19 (16%) of the 120 *S. maltophilia* isolates collected from three university hospitals in a particular region of Korea were found to be resistant to SXT. *S. maltophilia* isolates are often multidrug-resistant owing to the cumulative effects of intrinsic resistance and acquired resistance through integrons, transposons, and plasmids.

Recently, SXT resistance in *S. maltophilia* has been reported to be associated with the *sul1* gene carried by class 1 integrons [6, 7]. In addition, Toleman *et al.* [7] demonstrated that the ISCR elements linked to the *sul2* genes could mediate SXT resistance in *S. maltophilia*. Hu *et al.* [8] hypothesized that the *sul1* gene, if combined with the *dfrA* and *sul2* genes, could contribute to SXT resistance. Interestingly, some investigators have reported the detection of the *sul1* gene in SXT-susceptible *S. maltophilia* isolates; however, the incidence of *sul1* gene in the SXT-susceptible isolates was lower than that in the SXT-resistant isolates [8-10].

In this study, of the 32 SXT-resistant *S. maltophilia* isolates, 23 (72%) harbored the *sul1* gene. None of the SXT-susceptible *S. maltophilia* isolates were found to yield positive *sul1* PCR products. These results are similar to those of previous studies [6, 7]. It was not possible to detect the *sul1* gene in the nine isolates that demonstrated low-level SXT resistance, although the SXT MIC of the *sul1*-positive isolates was found to be higher, exhibiting a range of 64-128 mg/L (Table 1). Previous studies reported that *S. maltophilia* isolates harboring the *sul1* gene demonstrated high SXT MICs (>32 mg/L) [7, 8]. Accordingly, it is likely that high-level SXT resistance is associated with a specific and effective mechanism involving *sul1*.

In contrast to high-level resistance, low-level SXT resistance was not associated with the *sul* genes. Low-level resistance may result from a much broader variety of biochemical mechanisms

Table 1. Presence of *sul* genes and *Int11* and the corresponding association with trimethoprim/sulfamethoxazole (SXT) susceptibility

SXT susceptibility	MIC range (mg/L)	N of isolates	<i>sul1</i>	<i>sul1</i> linked <i>Int11</i>
Susceptible	≤0.06-2	220	0	ND
Resistant				
Low level	4-8	9	0	ND
High level	64-128	23	23 (100%)	15 (65%)

sul2 and *sul3* were not detected, regardless of susceptibility. Abbreviation: ND, not detected.

than those resulting in high-level resistance. Low antibiotic concentrations can select low-level antibiotic resistant variants, thus producing substantial stress in bacterial populations. This eventually influences the rate of genetic variation and the diversity of adaptive responses. The emergence of low-level resistance should be considered a warning signal, a hallmark of a possible evolutionary trend towards high-level, clinical resistance [16]. Further studies are needed in order to understand the mechanisms and significance of low-level SXT resistance in *S. maltophilia*.

Usually, the class 1 integrons harbor the *sul1* gene at the 3' end [6, 7]. However, there are several reports on *sul1*-positive isolates not being associated with class 1 integrons [8-10]. In this study, class 1 integrons were not detected in eight high-level SXT-resistant *sul1*-positive isolates.

The *sul2* gene has been reported to contribute to SXT resistance [7, 9]. However, in this study and in another previous Korean study, the *sul2* genes were not detected in the SXT-resistant *S. maltophilia* clinical isolates [10]. The *sul3* gene has not been associated with SXT-resistant *S. maltophilia* [7]. Contrary to the results reported by Tolman *et al.* [7], the ISCR elements and the *sul2* gene were not detected in this study. Consequently, the association of SXT resistance with the ISCR elements could not be confirmed or further elucidated in this study. Previous reports stated that the *dfrA* genes were identified in the SXT-resistant isolates and that the *sul* and *dfrA* genes could synergistically lead to high-level SXT resistance [8]. In the present study, we could not detect any *dfrA* genes. Whereas the presence of all types of the *dfrA* gene was not investigated, the primer pairs for the *dfrA* genes found in previous reports were included [8].

Previous studies reported the presence of the 3'-end of class 1 integrons, a semiconserved segment harboring the *qacEΔ1* and *sul1* genes, encoding resistance to quaternary ammonium compounds and sulfonamides, respectively [7-9]. In this study, the *qacEΔ1* genes along with the *sul1* genes were detected in nine (60%) class 1 integrons; however, six (40%) class 1 integrons harbored only the *int11* and *sul1* genes. The gene cassettes within the class 1 integrons included the aminoglycoside resistance genes *aac6'-Ib*, *aac6'-31*-like, *aacA7*, and *aacA7/aadA4a*.

In summary, the present results indicate that excessive antibiotic usage in clinical settings is selecting SXT-resistant *S. maltophilia* strains through horizontal gene transfer. Clinical microbiology laboratories need to carefully monitor, using continuous surveillance of SXT resistance rates, the possibility of *S.*

maltophilia acquiring SXT resistance from mobile elements. The *sul1* gene may play an important role in the mechanisms of high-level SXT resistance in *S. maltophilia*. Additionally, the *sul1* gene in the SXT-resistant *S. maltophilia* has been consistently associated with class 1 integrons. Although SXT resistance is uncommon in *S. maltophilia*, continuous monitoring of resistance trends is necessary to ensure appropriate antimicrobial therapy.

Authors' Disclosures of Potential Conflicts of Interest

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

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