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, sul1, Class 1 integron

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), because 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 TTT 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 CTG GGT TCT TTT AGC-3’], *sul3* [Forward, 5’-CAT TCT AGA AAA CAG TCG TAG TTT GGC TTT GGA-3’; Reverse, 5’-CAT CTG CAG CTA ACC TAG GGC TTT GGA-3’], and class 1 integron [Forward, 5’-GCC TGT CAT CAT CAT TTT CGG-3’; Reverse, 5’-CAT CTG CAG CTA ACC TAG GGC TTT GGA-3’], and class 1 integron [Forward, 5’-GCC TGT CAT CAT TTT CGG-3’; Reverse, 5’-GGG ATC ATC TAA CCC TCG GTC T-3’]; 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 *IntI1* and the corresponding association with trimethoprim/sulfamethoxazole (SXT) susceptibility

| SXT susceptibility | MIC range (mg/L) | N of isolates | *sul1* | *sul2* linked *IntI1* |
|--------------------|-----------------|---------------|-------|----------------------|
| 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 intI1 and sul1 genes. The gene cassettes within the class 1 integrons included the aminoglycoside resistance genes aac6¢-Ib, aac6¢-3I-like, aacA7, and aacA7/adcA4a.

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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