Class 1 Integrons as the Origin of Trimethoprim/Sulfamethoxazole (TMP/SMX)-Resistant *S. maltophilia* Isolates in Europe, Latin America and North America: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: TMP/SMX remains the treatment of choice for *S. maltophilia* (SM) infections, as this organism emerges as a more important nosocomial pathogen. Resistance (R) to TMP/SMX among SM is rare and other intrinsic R in this species limits combination therapies. Preliminary studies from Argentina suggest that the *sul1* determinant of TMP/SMX R resides on class 1 integrons and may effect TMP/SMX susceptibility. TMP/SMX R strains of SM (1998-2003) from the SENTRY Program were screened for this R determinant.
Methods: A total of 1,550 SM strains were processed by broth microdilution between 1998 and 2003, of which 2.0% were TMP/SMX R (MIC, > 2 µg/ml). An additional 277 strains from the Asia-Pacific region were included to assess the global resistance patterns. A total of 25 R strains were available for further susceptibility tests by repeat reference methods and Etest, epidemiologic typing (PFGE), and PCR using primers for Class 1 integrons containing *sul1* located as part of the 3'CS and the *sul2* gene, usually located on small plasmids. Medical centers in the USA, Brazil, Chile, Germany, Spain and Turkey had potential endemic/epidemic clusters.
Results: Alternative therapeutic agents among 17 tested drugs with % susceptible ≥ 30% were: polymyxin B (78.3%), gatifloxacin and levofloxacin (74.2%), ticarcillin/clavulanate (58.1%) and ceftazidime (38.7%). Two isolates in Chile had the same ribotype and PFGE pattern. PCR products for *sul1* on a class 1 integron were detected in 64% of strains, 5 with multiple genes (integron size between 0.4 and 4.5 kb). Nine strains (36%) carried the *sul2* resistance determinant.
Conclusions: R to TMP/SMX among SM was rare during 6 years of surveillance in North America, Latin America and Europe. The presence of *sul1* on a class 1 integron was a common finding and confers high-level R (> 32 µg/ml) to TMP/SMX. The presence of *sul2* was more common in Europe than in the Americas. Continued surveillance of this intrinsically R pathogen is needed due to the increasing isolation frequency among compromised patients.

INTRODUCTION

Bacterial resistance to trimethoprim/sulfamethoxazole is due to outer membrane impermeability, drug efflux, chromosomal mutations, plasmid acquisition and some species are capable of escaping the inhibitory effect of folate pathway antagonists. *Stenotrophomonas maltophilia* is an important pathogen because this species usually causes nosocomial infection among severely ill patients and has intrinsic antibacterial resistance mechanisms which typically present a multi-drug resistant phenotype. *S. maltophilia* is commonly resistant to ß-lactams ± ß-lactamase inhibitors, aminoglycosides and carbapenems. Fluoroquinolones and ticarcillin/clavulanate are active against some strains, but empiric therapy with these agents alone would not be advised. Trimethoprim/sulfamethoxazole remains an important therapeutic option for the treatment of infections caused by *S. maltophilia*. Although trimethoprim/sulfamethoxazole is currently the treatment of choice for this pathogen, resistance has emerged and dissemination has been documented.

It has been shown that integrons have contributed to the dissemination of plasmidic antimicrobial determinants among Gram-negative species. Recently, class 1 integrons have been found in *S. maltophilia* isolates from Argentina and Taiwan and can confer resistance to trimethoprim/sulfamethoxazole. This study will determine the level of trimethoprim/sulfamethoxazole-resistant *S. maltophilia* that have been evaluated by the SENTRY Antimicrobial Surveillance Program (1998 - 2003). Resistant strains from North and Latin America and Europe were evaluated to determine the presence of class 1 integrons and other resistance determinants.

MATERIALS AND METHODS

Organisms. During 1998 - 2003, 1,744 *S. maltophilia* isolates collected worldwide were forwarded to the SENTRY Antimicrobial Surveillance Program and tested for antimicrobial susceptibility. Among these isolates, 25 strains from different patients isolated in North and Latin America and Europe were confirmed resistant to trimethoprim/sulfamethoxazole. Identifications were confirmed using oxidase and all but one strain had a confidence identification percentage of > 90% using the Vitek System (bioMerieux, Hazelwood, MO).

Susceptibility methods. Isolates were tested for susceptibility using reference NCCLS broth microdilution methods in validated dry-form panels (TREK Diagnostics, Cleveland, OH). Further confirmation using trimethoprim/sulfamethoxazole Etests was performed according to manufacturer's directions (AB BIODISK, Solna, Sweden).

PCR experiments. The presence of Class 1 integrons in each strain was assessed using the Class 1 specific primers Int1F and sul1R designed to anneal to the 5' and 3' conserved sequences, respectively. Seven positive PCR products were chosen randomly, extracted from agarose gels after size separation and sequenced with IntF, QacR and custom made oligo primers.

The presence of *sul2* genes was similarly investigated with the *sul2* specific primers sul2F and sul2R; all nine positive products were further sequenced.

PCR reactions were carried out in 20µL final volume using 10µL of ABgene Expanded Hi-fidelity Master Mix (ABgene House, Surrey, UK). Primers were used at 10pM concentration and 1µL of overnight bacterial culture at OD 1.0 at 600 nm was used as a template. The cycling parameters were: 95°C for five minutes followed by 30 cycles of 95°C for one minute, annealing at 55°C for one minute and extension 68°C ranging from one to four minutes and ending with five minute incubation at 68°C.

RESULTS

- There was no significant increase in resistance among *S. maltophilia* during 1998 - 2003 for the fluoroquinolones, cephalosporins and trimethoprim/sulfamethoxazole (Table 1). Resistance increased for piperacillin/tazobactam and ticarcillin/clavulanate with highest resistance rates in 2003 at 65.0% and 22.5%, respectively.

- Among the fluoroquinolones, gatifloxacin and levofloxacin were equally active and typically resistance rates ranged between 4 - 11% each year (Table 1) while ciprofloxacin activity was poor (16.2 - 36.5% susceptibility rates).

- Table 2 lists the alternative agents for treating *S. maltophilia* resistant to trimethoprim/sulfamethoxazole. Only fluoroquinolones including gatifloxacin and levofloxacin (76.0% susceptible), polymyxin B (78.9% susceptible) and ticarcillin/clavulanate (60.0% susceptible) showed modest activity against these isolates.

- Among the 25 trimethoprim/sulfamethoxazole isolates, one to three PCR products with integron primers were detected in 16 strains (64%) with size(s) ranging from 0.4 to 4.5 Kb (Table 3). The *sul2* gene was amplified by PCR and sequenced in nine strains (36%), and was more frequently found in strains from Europe (six out of 10).

- Transfer of trimethoprim/sulfamethoxazole-resistant *S. maltophilia* between two patients in Chile was confirmed during this study (Table 3, strain numbers 9189A and 12221A). These strains were isolated over a year apart, suggesting that mobile elements (*sul1*) can persist within the hospital setting.

Table 1. Activity of nine antimicrobial agents tested against 1,744 isolates of *S. maltophilia* from SENTRY Program participants in North America, Latin America, Europe and the Asia-Pacific regions during 1998 - 2003.

Antimicrobial agent	Year (% susceptible/resistant)[a]					
	1998[b]	1999	2000	2001	2002	2003
Trimethoprim/Sulfamethoxazole	94.1/5.9	96.3/3.7	95.8/4.2	96.6/3.4	96.6/3.4	97.0/3.0
Gatifloxacin	78.2/8.5	86.4/7.9	89.8/4.9	89.6/3.7	87.5/5.7	82.3/7.3
Levofloxacin	78.2/11.4	86.8/7.9	89.8/4.5	89.3/4.2	86.9/6.6	83.2/7.3
Ciprofloxacin	16.2/48.7	28.5/45.9	25.0/45.5	36.5/33.9	32.5/37.9	24.6/41.0
Ticarcillin/Clavulanate	62.7/14.8	49.2/17.8	52.5/17.3	49.3/14.1	45.0/19.4	49.1/22.5
Piperacillin/Tazobactam	25.7/50.2	16.1/57.0	9.8/56.6	11.3/59.9	14.2/62.1	10.3/65.0
Ceftazidime	48.0/36.9	44.6/38.4	50.6/36.2	54.6/33.9	51.9/31.6	51.3/37.1
Cefepime	22.1/42.8	26.9/45.5	22.3/46.8	28.2/43.1	29.9/39.6	22.0/44.8
Amikacin	11.1/84.1	14.5/72.3	20.0/64.5	12.8/76.5	9.7/74.9	12.1/80.6

a. Percent susceptible/resistant was based upon NCCLS recommended breakpoints (M100-S14).
b. Excludes European strain data during 1998 only.

Table 2. Antibiograms of 17 alternative agents for trimethoprim/sulfamethoxazole-resistant *S. maltophilia* (25 strains).

Antimicrobial agent	MIC (µg/ml)			% by category[a]	
	Range	50%	90%	Susceptible	Resistant
Amikacin	8->32	>32	>32	4.0	88.0
Gentamicin	2->8	>8	>8	4.0	92.0
Tobramycin	2->16	>16	>16	4.0	88.0
Aztreonam	1->16	>16	>16	8.0	92.0
Ceftazidime	≤2->16	16	>16	36.0	28.0
Cefepime	2->16	16	>16	8.0	52.0
Ciprofloxacin	0.5->2	2	>2	12.0	48.0
Gatifloxacin	0.12->4	1	>4	76.0	16.0
Levofloxacin	0.25->4	1	>4	76.0	12.0
Piperacillin	32->128	128	>128	0.0	80.0
Piperacillin/Tazobactam	8->64	>64	>64	12.0	52.0
Ticarcillin	≤1->128	128	>128	12.0	56.0
Ticarcillin/Clavulanate	≤1->128	16	128	60.0	20.0
Tetracycline	8->8	>8	>8	0.0	80.0
Imipenem	>8	>8	>8	0.0	100.0
Meropenem	1->8	>8	>8	12.0	78.0
Polymyxin B (19)	≤1->8	≤1	8	78.9	21.1

a. Based upon NCCLS recommended breakpoints (M100-S15).

Table 3. Molecular analysis of 25 trimethoprim/sulfamethoxazole (TMP/SMX)-resistant *S. maltophilia* isolates and three negative controls.

Year	Strain no.	Country	TMP/SMX MIC (µg/ml)[a]	Integron size(s)	PCR/sequence results	
					sul1[b]	*sul2*
2001	1113I[c]	USA	1	-	-	-
2002	1696D[c]	USA	1	-	-	-
1998	6147A[c]	Venezuela	2	-	-	-
1998	2170C	USA	>32	-	-	+
2001	867C	USA	>32	-	-	-
2001	489I	USA	>32	1Kb	+	-
2000	345C	Canada	>32	1.4Kb	+	-
1998	7666A	Argentina	>32	4.5Kb	+	-
2000	9189A[d]	Chile	>32	1Kb	+	-
2001	12221A[d]	Chile	>32	1Kb	+	-
2002	3444A	Mexico	>32	3Kb	+	-
2003	12357A	Brazil	>32	0.4Kb, 1.2Kb,1.5 Kb	+	-
2002	14469A	Brazil	>32	1Kb	+	-
2003	9431A	Brazil	>32	0.4Kb, 1Kb	+	-
2001	7618A	Venezuela	>32	4Kb	+	-
2002	3932C	Brazil	>32	4.5Kb	+	+
2003	98I	Brazil	>32	1Kb	+	-
2002	4647C	Brazil	>32	-	-	+
2002	4225C	Belgium	>32	-	-	+
2001	12044A	Spain	>32	-	-	+
2001	12049A	Spain	>32	0.4Kb, 3Kb	+	-
2000	2139C	Turkey	>32	-	-	+
2001	3800C	Turkey	>32	-	-	+
2001	14263A	Turkey	>32	1Kb	+	-
2000	2597C	Italy	>32	1.3Kb, 1.5Kb	+	-
2002	1893A	Germany	>32	0.4Kb, 0.9Kb	+	-
2001	5232C	Germany	>32	-	-	+
2001	12876A	France	>32	-	-	+

a. MIC results were obtained using Etest strips.
b. Integron PCR products were initially screened using primers targeting qacEΔ1 (adjacent to *sul1*) and confirmed with a primer located within *sul1*.
c. Negative control strains.
d. Isolates shared the same pulsed field gel electrophoresis pattern.

CONCLUSIONS

- Isolation of trimethoprim/sulfamethoxazole-resistant *S. maltophilia* strains remained rare during the 1998 - 2003 surveillance years. Nearly all isolates were genetically unrelated and occurred in all regions surveyed. The higher resistance rates were noted in Europe and the Asia-Pacific region compared to the American continents.

- Treatment options for infections caused by *S. maltophilia* are limited, with trimethoprim/sulfamethoxazole (drug-of-choice) and the newer fluoroquinolones being the only highly active agents remaining. Ticarcillin/clavulanate susceptibility has decreased to < 50% among contemporary isolates monitored by the SENTRY Program.

- Strains of *S. maltophilia* with high-level resistance to trimethoprim/sulfamethoxazole (> 32 µg/ml) were isolated in North America, Latin America and Europe. Isolates in the Americas generally carried *sul1* on integrons, and European isolates were more likely to have the *sul2* resistance determinant which is usually located on small plasmids.

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