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Current Situation of Antimicrobial Resistance and Genetic Differences in *Stenotrophomonas maltophilia* Complex Isolates by Multilocus Variable Number of Tandem Repeat Analysis

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Background: *Stenotrophomonas maltophilia* is one of several opportunistic pathogens of growing significance. Several studies on the molecular epidemiology of *S. maltophilia* have shown clinical isolates to be genetically diverse.

Materials and Methods: A total of 121 clinical isolates tentatively identified as *S. malophilia* from seven tertiary-care hospitals in Korea from 2007 to 2011 were included. Species and groups were identified using partial *gyr*B gene sequences and antimicrobial susceptibility testing was performed using a broth microdilution method. Multi locus variable number of tandem repeat analysis (MLVA) surveys are used for subtyping.

Results: Based on partial *gyr*B gene sequences, 118 isolates were identified as belonging to the *S. maltophilia* complex. For all *S. maltophilia* isolates, the resistance rates to trimethoprime-sulfamethoxazole (TMP/SMX) and levofloxacin were the highest (both, 30.5%). Resistance rate to ceftazidime was 28.0%. 11.0% and 11.9% of 118 *S. maltophilia* isolates displayed resistance to piperacillin/tazobactam and tigecycline, respectively. Clade 1 and Clade 2 were definitely distinguished from the data of MLVA with amplification of *loci*. All 118 isolates were classified into several clusters as its identification.

Conclusion: Because of high resistance rates to TMP/SMX and levofloxacin, the clinical laboratory department should consider providing the data about other antimicrobial agents and treatment of S. *maltophilia* infections with a combination of antimicrobials can be considered in the current practice. The MLVA evaluated in this study provides a fast, portable, relatively low cost genotyping method that can be employed in genotypic linkage or transmission networks comparing to analysis of the *gyr*B gene .

Key Words: *Sternotrophomonas maltophilia;* Trimethoprime-sulfamethoxazole; Multilocus variable number of tandem repeat analysis

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Introduction

Stenotrophomonas maltophilia is one of several opportunistic pathogens of growing significance and is one of the most common nonfermentative Gram-negative bacillus isolated from clinical specimens [1, 2]. The British Society for Antimicrobial Chemotherapy (BSAC, version 10.2, 2011) recommends disk diffusion and dilution testing for trimethoprim-sulfamethoxazole (TMP/SMX) only, while the Clinical and Laboratory Standards Institute (CLSI) recommends dilution testing for TMP/SMX, ceftazidime, chloramphenicol, levofloxacin, minocycline, and ticarcillin-clavulanate, and disk diffusion testing for only TMP/SMX, levofloxacin, and minocycline [3].

Several studies on the molecular epidemiology of *S. maltophilia* have shown clinical isolates to be genetically diverse [3, 4]. Genotypic profiles have been determined by various methods, including restriction fragment length polymorphism analysis of the *gyr*B gene or the intergenic region between *sme*D and *sme*T genes, amplified fragment length polymorphism fingerprinting, Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR), Multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE) analysis [5, 6]. Changes in the number of repeats among isolates can be checked by multilocus variable number of tandem repeat analysis (MLVA) surveys are used for subtyping purposes [5, 7]. MLVA assays provide results that parallel PFGE data [5]. The MLVA technique involves amplification and size analysis of polymorphic DNA regions containing variable numbers of tandem repeated sequences, and is an established method to classify isolates of microbial species [8].

S. maltophilia GTAG (SMAGs) make up approximately 0.5% of the K279a genome, and are spread throughout the chromosome either as single units, or in pairs, separated by 5–80 bp long spacers [5]. The size of the SMAG family is shown for repetitive extragenic palindromic sequences [5]. SMAGs are reiterated in tandem at multiple chromosomal *loci*, along with tracts of variable length of DNA. The occurrence of SMAG arrays to set up PCR-based typing protocols, and comparison of the electrophoresis sizing observations was by DNA sequencing of selected PCR products.

Studies on the antimicrobial susceptibility of *S. maltophilia* treated with various antimicrobial agents including TMP/SMX, ceftazidime, chloramphenicol, levofloxacin, minocycline, piperacillin-tazobactam, ticarcillin-clavulanate, and tigecycline have been performed [3, 4, 9-12]. Infections caused by *S. maltophilia* are particularly difficult to manage because they show resistance to many classes of antimicrobial agents [9-13]. However, only two studies have focused on the antimicrobial susceptibility of *S. maltophilia* in Korea [3, 14].

The aim of this study was to determine the antimicrobial susceptibility of recent clinical *S. maltophilia* complex isolates from Korea and the clonality of the clinical *S. maltophilia* should be assessed in order to detect genotype relationships. This study was undertaken to determine whether the strains could be rapidly and accurately genotyped with MLVA.

Materials and Methods

1. Bacterial isolates

A total of 121 clinical isolates tentatively identified as *S. malophilia* were included in this study. They were collected from seven tertiary-care hospitals in Korea from 2007 to 2011 and were identified conventionally using VITEK2 systems (bioMérieux, Inc., Haselwood, MO, USA) in the hospitals' clinical microbiology laboratories. Among them, 85 isolates were from blood, and the others were from sputum (9 isolates), urine (8 isolates), endotracheal aspirate (5 isolates), transtracheal aspirate (5 isolates), bile (3 isolates), pericardial fluid (2 isolates), pus (2 isolates), and discharge from ear (1 isolate). The source of one isolate was unknown. 98 isolates were from intensive care unit.

2. Species identification

To identify the *Stenotrophomonas* spp. isolates, we determined the partial *gyrB* gene sequence of all isolates using XgyrB1F/XgyrB1R (5'-ACGAGTACAACCCGGACAA-3' / 5'-CCCATCARGGTGCTGAAGAT-3'), which amplifies one of the variable regions of the *gyr*B gene, region 2 [15, 16]. Ambiguous *gyr*B gene sequences from three isolates were excluded in further analyses. We published these results previously [15].

3. Antimicrobial susceptibility testing

In vitro susceptibility testing was performed with 118 isolates identified as belonging to the S. maltophila complex in this study using the broth microdilution method according to the CLSI guidelines for five antimicrobial agents including ceftazidime, levofloxacin, piperacillin/tazobactam, TMP/SMX, and tigecycline [17]. The broth microdilution method always tends to give slightly higher minimum inhibitory concentrations (MICs), indicating its potency in the identification of isolates resistant to TMP/SMX because of trailing endpoints with TMP/SMX in the broth microdilution method [18]. TMP/

SMX was tested by using the standard agar dilution method according to the CLSI guidelines [12]. The interpretive criteria used were those established in the CLSI standard M100-S21 [19]. Regarding tigecycline, interpretive criteria were defined based on the USA-FDA breakpoint criteria for *Enterobacteriaceae* (susceptible ≤ 2 mg/L, intermediate 4 mg/L, and resistant ≥8 mg/L). *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as control strains. Multidrug resistant (MDR) isolate was defined as one showing resistance to two or more antimicrobial agents [1].

4. Multilocus variable number of tandem repeat analysis (MLVA)

The DNA of single colonies derived from the final subcultures was analyzed by PCR amplification of DNA regions of interest. Genomic DNA was extracted and PCR reactions were carried out by incubating 20 ng of DNA with 160 ng of each primer in the presence of dXTPs (200 nanomoles), 1.5mM magnesium chloride and the Taq DNA polymerase Recombinant (Invitrogen, Midland, ON, Canada). Because of the high GC content of the *S. maltophilia* genome (> 66%), all PCR reactions were carried out in GC-rich buffer (Roche, Alameda, CA, USA) [5]. The oligomers used as primers, and the annealing temperatures, are from the paper by Emanuela Roscetto et al, 2008 [5]. Samples were incubated at 95°C for 5 minutes, and subsequently for 1 minute at 95°C, 1 minute at the annealing temperature and 1 minute at 72°C, for a total of 3 cycles. At the end of the cycle, samples were kept at 72°C for 7 minutes before harvesting. PCR products were electrophoresed on 1.5–2% agarose gels in 0.5 × TBE buffer (45 mM Tris pH 8, 45 mM Borate, 0.5 mM EDTA) at 120 V (constant voltage). The 100 bp ladder (Fermentas, Carlsbad, CA, USA) was used as molecular weight marker [13]. PCR products were resolved by agarose electrophoresis in 1.5 % multipurpose agarose gel (Fermentas, France), stained with ethidium bromide and photographed under UV light (BioRad, Berkeley, CA, USA).

PCR fragment length estimation of the SMAGs alleles was by reference to a 100-bp DNA size standard. Confirmation of the electrophoresis sizing observations was by DNA sequencing of selected PCR products.

5. Data analysis and statistics

Cluster analysis of the MLVA typing data was performed in PHYLOVIZ softwear (downloading from http://www.phyloviz.net/) with the results of amplification and size analysis of polymorphic DNA regions containing variable numbers of tandemly repeated sequences of *S. maltophilia* complex isolates. Data were analyzed using SPSS 11.0 for Windows 2000 (SPSS, Inc., Chicago, IL, USA). Categorical data were tested using Chi-square analysis. Differences were considered statistically significant at a *P*-value of <0.05 for all tests.

Results

1. Antimicrobial susceptibility testing

Table 1 shows the MICs of antimicrobial agents and the resistance rates of the *S. maltophilia* complex isolates tested. For all *S. maltophilia* complex isolates, the resistance rates to TMP/SMX and levofloxacin were the highest (both were 30.5%) among five antimicrobial agents (Table 1). MIC_{50} and MIC_{90} of TMP/SMX were 2/38 and 32/608 mg/L, respectively. Resistance rate to ceftazidime was 28.0%. 11.0% and 11.9% of 118 *S. maltophilia* complex isolates displayed resistance to piperacillin/tazobactam and tigecycline, respectively. Thirty-eight MDR isolates (32.2%) were identified.

2. Multilocus variable number of tandem repeat analysis (MLVA)

Considering previous identification of *S. maltophilia* complex, there were scattered clustering of *S. maltophilia* complex without relationship to identification in all data from amplification of 12 selected SMAGs *loci*, because the results showed that the majority of the isolates were not amplified successfully in *loci* I, IV, VI, and X. The number of alleles at each of the *loci* ranged from 0 to 21 numbers of allele repeat units on the 118 isolates analyzed (Table 2). The clonal profiles of the 118 *S. maltophilia* isolates were determined by

Table 1. Antimicrobial activity of antimicrobial agents against the 118 tested clinical *Stenotrophomonas maltophilia* complex isolates.

Agent	MIC (μg/mL)		Number of isolates (%)	
	MIC_{50}	MIC_{90}	Susceptible	Resistant
Ceftazidime	8	64	70 (59.3)	33 (28.0)
Levofloxacin	2	16	61 (51.7)	36 (30.5)
TMP/SMX	2/38	32/608	82 (69.5)[a] 65 (55.1)	36 (30.5)[a] 53 (44.9)
TZP	16/4	128/4	84 (71.2)	13 (11.0)
Tigecycline	2	8	83 (70.3)	14 (11.9)
MDR			38 (32.2)	

[a]Results from standard agar dilution methods.
MIC, minimum inhibitory concentration; TMP/SMX, trimethoprim-sulfamethoxazole; TZP, piperacillin/tazobactam; MDR, multidrug resistance

Table 2. MLVA data from amplification of 12 selected SMAGs loci of *Stenotrophomonas maltophilia*

No.	Classification No.	*gyr*B	Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1	12001	*Spav*	*S. pavanii*		2	4		4		1	0	4		0	3
2	21133	*Smal I*	*S. maltophilia I-2*	6	1	1	1	3	12	4	1	5	4	5	3
3	Ambiguous *gyr*B gene sequences														
4	21201	*Smal I*	*S. maltophilia I-2*	11	1	1	2	3	10	4	1	5	6	5	2
5	21101	*Smal I*	*S. maltophilia I-1*	6	1	2	3	4	11	3	3	5	3	5	3
6	11101	*Smal II*	*S. maltophilia II-1*		2	3		0		1	0	1		1	1
7	13001	*Smal III*	*S. maltophilia III*		2	1		2	0	4	1	2		2	1
8	21102	*Smal I*	*S. maltophilia I-1*		1	2		4		3	0	2		4	2
9	21103	*Smal I*	*S. maltophilia I-1*	11	1	2	3	4	12	4	1	6	18	4	3
10	21104	*Smal I*	*S. maltophilia I-1*		1	2		4		3	0	2		4	3
11	14001	*Pbet*	*P. beteli*	1	2	0		2	0	0	1	2		0	0
12	13002	*Smal III*	*S. maltophilia III*		2	1		0		15	0	1		1	1
13	21105	*Smal I*	*S. maltophilia I-1*		1	2		4		2	0	3		3	3
14	22001	*Pgen*	*P. geniculata*	1	1	0		2	0	3	1	2	2	2	1
15	21106	*Smal I*	*S. maltophilia I-1*	8	1	2	4	4	3	3	3	5	5	5	3
16	21107	*Smal I*	*S. maltophilia I-1*		1	2		2		3	0	2		4	3
17	11301	*Smal II*	*S. maltophilia II-3*		3	4		0		1	0	4		1	2
18	11102	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	2
19	21202	*Smal I*	*S. maltophilia I-2*		1	1		2		2	1	3		4	2
20	11103	*Smal II*	*S. maltophilia II-1*		2	3		0		1	0	2		1	4
21	21108	*Smal I*	*S. maltophilia I-1*	2	1	2	2	3	12	2	1	1	4	1	3
22	13003	*Smal III*	*S. maltophilia III*		2	1		0		21	0	1		1	1
23	12002	*Spav*	*S. pavanii*		2	4		3		1	0	4		4	3
24	12003	*Spav*	*S. pavanii*		2	4		3		3	0	3		5	2
25	21109	*Smal I*	*S. maltophilia I-1*	3	1	2	0	2	10	2	2	1	1	5	2
26	11201	*Smal II*	*S. maltophilia II-2*		2	2		0		1	0	1		1	1
27	12004	*Spav*	*S. pavanii*		2	4		3		1	0	4		0	3
28	11104	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	2
29	11105	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	2
30	11302	*Smal II*	*S. maltophilia II-3*	4	3	4	1	4	0	3	1	2		2	4
31	11106	*Smal II*	*S. maltophilia II-1*		2	3	2	2	4	1	1	1		2	4
32	23001	*Phib*	*P. hibisciola*	2	1	0		2	2	3	2	2	4	2	1
33	11107	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	2		1	4
34	13004	*Smal III*	*S. maltophilia III*		2	1		0		15	0	1		1	1
35	12005	*Spav*	*S. pavanii*		2	4		2		1	0	3		4	3
36	11202	*Smal II*	*S. maltophilia II-2*		2	2		0		1	0	1		1	3
37	21110	*Smal I*	*S. maltophilia I-1*	2	1	2	2	4	12	3	1	1	4	1	4
38	11108	*Smal II*	*S. maltophilia II-1*	2	2	3	1	4	3	1	1	1	4	2	4
39	12006	*Spav*	*S. pavanii*		2	4		3		1	0	4		4	3
40	21111	*Smal I*	*S. maltophilia I-1*		1	2		3		3	0	1		2	3
41	12007	*Spav*	*S. pavanii*		2	4		4		3	0	3		7	5
42	11109	*Smal II*	*S. maltophilia II-1*	5	2	3	2	3	4	1	1	1	4	2	4
43	12008	*Spav*	*S. pavanii*		2	4		1		2	0	4		6	2
44	21112	*Smal I*	*S. maltophilia I-1*		1	2		4		5	0	2		3	2
45	21113	*Smal I*	*S. maltophilia I-1*		1	2		4		5	1	4		3	3
46	11110	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	2		1	4
47	12009	*Spav*	*S. pavanii*		2	4		1		3	0	4		6	2
48	21203	*Smal I*	*S. maltophilia I-2*		1	1		2		4	1	2		4	3
49	11111	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	2		1	4
50	12010	*Spav*	*S. pavanii*		2	4		1		3	0	4		5	3

Table 2. Continued.

No.	Classification No.	*gyr*B	Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
51	11112	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	2		1	4
52	11113	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	1
53	21114	*Smal I*	*S. maltophilia I-1*		1	2		2		4	0	2		4	3
54	21115	*Smal I*	*S. maltophilia I-1*		1	2		2		2	0	1		4	1
55	11203	*Smal II*	*S. maltophilia II-2*		2	2		0		1	0	1		1	1
56	22002	*Pgen*	*P. geniculata*		1	0		2	0	3	1	1		1	2
57	21116	*Smal I*	*S. maltophilia I-1*		1	2		2		2	1	2		2	3
58	11303	*Smal II*	*S. maltophilia II-3*		3	4		0		2	0	1		2	1
59	21117	*Smal I*	*S. maltophilia I-1*		1	2		2		2	0	1		4	1
60	11114	*Smal II*	*S. maltophilia II-1*	5	2	3	2	4	4	1	1	1	4	2	4
61	21118	*Smal I*	*S. maltophilia I-1*	2	1	2	1	3	10	3	2	1	1	5	3
62	26001	*Smal IV*	*B0811-107*	3	1	1		2	0	3	2	2		0	1
63	11115	*Smal II*	*S. maltophilia II-1*		2	3	2	5	4	1	1	1		2	4
64	11116	*Smal II*	*S. maltophilia II-1*		2	3	2	2	4	1	1	1	4	2	4
65	21119	*Smal I*	*S. maltophilia I-1*	11	1	2	2	4	3	4	3	5	5	5	4
66	11204	*Smal II*	*S. maltophilia II-2*	2	2	2	2	2	0	2	1	2	4	2	1
67	21120	*Smal I*	*S. maltophilia I-1*	1	1	2	2	4	12	4	1	1	4	1	3
68	22003	*Pgen*	*P. geniculata*	1	1	0		2	0	3	1	2	4	2	1
69	21204	*Smal I*	*S. maltophilia I-2*	4	1	1	1	3	10	5	1	5	5	5	3
70	22004	*Pgen*	*P. geniculata*	2	1	0		2	0	4	1	2	2	2	1
71	12011	*Spav*	*S. pavanii*		2	4		3		2	0	4		5	3
72	12012	*Spav*	*S. pavanii*		2	4		1		2	0	4		6	3
73	11205	*Smal II*	*S. maltophilia II-2*	2	2	2	2	2	0	3	1	2		2	1
74	11117	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	2		1	4
75	21205	*Smal I*	*S. maltophilia I-2*	5	1	1	1	2	10	6	2	5	4	5	4
76	12013	*Spav*	*S. pavanii*	2	2	4		4	6	4	0	7		9	3
77	13005	*Smal III*	*S. maltophilia III*	2	2	2		2	0	4	1	2		2	1
78	11206	*Smal II*	*S. maltophilia II-2*		2	2		0		1	0	1		1	1
79	12014	*Spav*	*S. pavanii*		2	4		0		2	0	4		5	3
80	21121	*Smal I*	*S. maltophilia I-1*	3	1	2	0	2	10	3	3	1	1	5	2
81	21122	*Smal I*	*S. maltophilia I-1*	7	1	2	3	5	12	6	1	1		4	3
82	13006	*Smal III*	*S. maltophilia III*		2	1		1		4	0	1		1	1
83	13007	*Smal III*	*S. maltophilia III*		2	1		0		3	0	1		1	
84	25001		*08-B-253*		1	1		0		1	2	0		0	0
85	21123	*Smal I*	*S. maltophilia I-1*	7	1	2	3	4	1	5	3	1	5	4	2
86	11118	*Smal II*	*S. maltophilia II-1*		2	3		0		1	0	2		0	4
87	ambiguous *gyr*B gene sequences									3				4	
88	12015	*Spav*	*S. pavanii*		2	4		1		3	0	4		6	3
89	21124	*Smal I*	*S. maltophilia I-1*	7	1	2	0	4	4	4	3	0	1	5	3
90	12016	*Spav*	*S. pavanii*		2	4		1		3	0	4		4	3
91	12017	*Spav*	*S. pavanii*		2	4		3		3	0	4		1	3
92	21125	*Smal I*	*S. maltophilia I-1*	7	1	2	0	4	3	2	3	1	18	4	3
93	14002	*Pbet*	*P. beteli*	2	2	0		2	0	0	3	2		0	0
94	15001		*K01-43*	2	2	0		2	0	1	2	2		0	3
95	11119	*Smal II*	*S. maltophilia II-1*		2	3		0		1	0	1		2	1
96	21126	*Smal I*	*S. maltophilia I-1*		1	2		3		3	2	2		1	3
97	11120	*Smal II*	*S. maltophilia II-1*	3	2	3	2	2	3	1	1	2		2	5
98	21206	*Smal I*	*S. maltophilia I-2*	7	1	1	4	2	7	5	1	1		5	4
99	22005	*Pgen*	*P. geniculata*	10	1	0	0	9	0	4	1	2		4	2
100	11121	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	1

Table 2. Continued.

No.	Classification No.	*gyr*B	Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
101	11122	*Smal II*	*S. maltophilia II-1*		2	3	2	2	4	1	1	2		2	1
102	11123	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		0	1
103	22006	*Pgen*	*P. geniculata*	2	1	0		2	0	4	1	2		2	1
104	ambiguous *gyr*B gene sequences				-5										
105	24001	*Sarf*	*S. africana*	2	1	2		2	0	4	1	2		2	1
106	21127	*Smal I*	*S. maltophilia I-1*	2	1	2	2	2	7	4	1	6	5	1	3
107	11124	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	1
108	21128	*Smal I*	*S. maltophilia I-1*		1	2		2		3	1	1		1	3
109	21129	*Smal I*	*S. maltophilia I-1*	8	1	2	3	5	6	6	1	3	5	4	2
110	22007	*Pgen*	*P. geniculata*	2	1	0		2	0	3	1	2	4	2	1
111	11125	*Smal II*	*S. maltophilia II-1*		2	3	1	2	4	1	1	1	4	2	4
112	22008	*Pgen*	*P. geniculata*	1	1	0	1	2	0	3	1	2	4	2	1
113	11126	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	1
114	11127	*Smal II*	*S. maltophilia II-1*		2	3		1		1	0	1		1	2
115	12018	*Spav*	*S. pavanii*		2	4		1		3	0	4		5	3
116	22009	*Pgen*	*P. geniculata*	1	1	0		2	0	3	1	2	3	2	1
117	21130	*Smal I*	*S. maltophilia I-1*		1	2		3		5	0	2		2	4
118	21131	*Smal I*	*S. maltophilia I-1*	2	1	2	1	2	7	4	1	3	2	1	3
119	22010	*Pgen*	*P. geniculata*	1	1	0		2	0	3	1	2		2	1
120	21132	*Smal I*	*S. maltophilia I-1*	2	1	2	1	3	7	4	1	3	2	1	3
121	11128	*Smal II*	*S. maltophilia II-1*		2	3		2		1	0	2		1	4

The PCR products derived from the amplification of loci I to XII in the listed strains are labeled with the number of SMAG repeats present.
MLVA, multi locus variable number of tandem repeat analysis; SMAG, *Sternotrophomonas maltophilia* GTAG; PCR, polymerase chain reaction.

random primer PCR fingerprinting and divided into two groups (clade A and B) as described previous study [15]. Antimicrobial resistance rates were varied by species or groups of *S. maltophilia* complex. Isolates of Clade A showed significantly lower antimicrobial resistance rates than those of Clade B as previous study [15]. Clade A and Clade B were also definitely distinguished from the data of MLVA with amplification of *loci*. All 118 isolates were classified into several clusters as its identification (Fig. 1).

Considering previous identification of *S. maltophilia* complex, there were also scatterred clustering of *S. maltophilia* complex with relationship to identification in all data from eight fully amplified *loci* II, III, V, VII, VIII, IX, XI and XII. All *Pseudomonas geniculata* were from one single hospital from previous identification study (Red arrow in Fig. 1). With hierarchical clustering, the MLVA profiles of the members of the 118 *S. maltophilia* complex were classified in all data from twelve SMAGs *loci*.

Discussion

TMP/SMX alone, or in combination with other agents is still considered the treatment of choice for suspected or culture-proven *S. maltophilia* infections. Resistance rates to TMP/SMX have been reported to vary geographically [1-3, 14, 19], but were generally less than 20%. However, isolates from cystic fibrosis patients and from patients in some Asian countries, such as Taiwan, as well as Turkey, showed high resistance rates (31.3-100%) [19, 20]. The most notable finding in this study is the high resistance rate to TMP/SMX. Worldwide surveillance studies have shown relatively low resistance rates, varying from 4% to 20% [1-3, 12, 14, 20]. A recent report from a Korean hospital also documented a low resistance rate of 6% to TMP/SMX using the agar dilution method [3]. For all clinical *S. maltophilia* complex isolates, the resistance rate to TMP/SMX was 45.5% with the broth microdilution test and 29.8% with the agar dilution test, which was an unexpected result. We re-confirmed that the broth microdilution method always tends to give slightly higher MICs, indicating its potency in identifying isolates resistant to TMP/SMX because of trailing endpoints with TMP/SMX in the broth microdilution method [18]. So, the broth microdilution method can be somewhat useful in clinical practice to detect potential resistant isolate. In the agar dilution test, the resistance rate to TMP/SMX was 30.5%. This rate was much higher than the rate

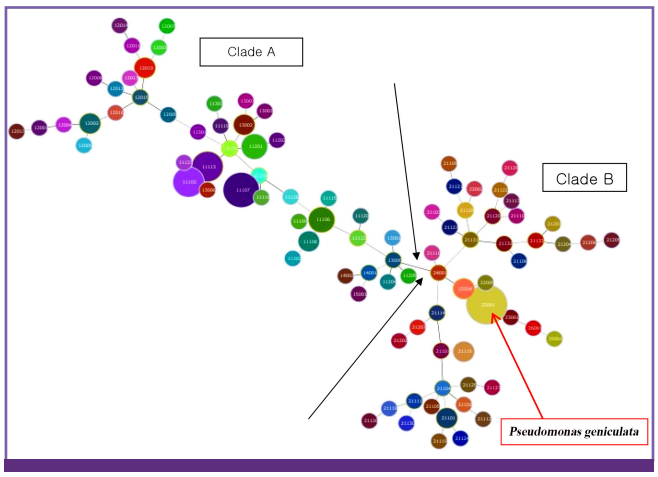

Figure 1. Dendrogram demonstrating genetic disimilarity of 118 *Stenotrophomonas maltophilia* complex isolates based on MLVA genotyping method in amplifications of all eight SMAGs loci.

in previous studies, although the patient group in this study did not include patients with cystic fibrosis who usually show high resistance rates to TMP/SMX according to previous studies. The resistance rate to levofloxacine was 30.5%, which was higher than the rates of 3 to 13% seen in a previous studies [3, 12-14, 20-22]. The tetracycline derivatives minocycline and tigecycline have shown good *in vitro* activity against clinical isolates of *S. maltophilia* [3]. Previous studies in Taiwan, Brazil, Spain, and the USA showed that there was no or low resistance to tigecycline [3, 20-22]. However, this study showed that 11.9% of clinical 118 *S. maltophilia* complex isolates displayed resistance to tigecycline. The high resistance rates to TMP/SMX, levefloxacin, and tigecycline in this study should be noted.

The isolates showed low rates of susceptibilities (23 to 43%) to ceftazidime, ticarcillin-clavulanic acid, and piperacillin/tazobactam [23]. However, susceptibility rates to piperacillin/tazobactam and ceftazidime in this study were 71.2% and 59.3%, respectively, which were higher than the susceptibility rates in other studies [2, 3].

As a whole, the most notable finding in this study is the high resistance rate to TMP/SMX. The differences in the TMP/SMX resistance rates between studies in Korea may be due to differences in tertiary hospitals, isolation periods, and numbers of isolates. In addition, most of our isolates (98 isolate) were from intensive care units. The high TMP/SMX resistance rate found in this study is of concern because the preferred treatment option of *S. maltophilia* infections is TMP/SMX [1]. Thus, continuous surveillance of antimicrobial resistance of *S. maltophilia* is recommended. In contrast to the high resistance to TMP/SMX and levofloxacin, piperacillin/tazobactam and tigecycline showed potent activities against *S. maltophilia* complex isolates. The resistance rate to the tigecycline was 11.9%, which was the highest resistance rate in the world. This relatively high resistance rate may lead to tigecycline being considered an alternative therapeutic option as a component of combination therapy [1, 2]. Treatment strategies have included the use of select antibiotics in synergy [1]. Piperacillin/tazobactam can be a candidate for combination therapy in treating *S. maltophilia* because isolates show a high susceptibility rate to this combination. However, piperacillin/tazobactam cannot be used as monotherapeutic drugs to treat *S. maltophilia* because this microorganism has a high intrinsic resistance to most penicillins and cephalosporins, as well as to all carbapenems. Treatment of *S. maltophilia* infections with a combination of two or three antimicrobials can be considered in the current practice because *S. maltophilia* has a high resistance rate to TMP/SMX and levofloxacin. In clinical practice, the clinical laboratory department usually provides information about susceptibility to TMP/SMX and levofloxacin. Physicians usually choose one of these agents or both agents. Therefore, the clinical laboratory department should consider providing the data about other antimicrobial agents such as ceftazidime, piperacillin/tazobactam, and tigecycline to physicians.

We evaluated an MLVA to assess the molecular epidemiology of *S.maltophilia* complex. Studies based on MLST demonstrated that *S. maltophilia* isolates were heterogeneous, because several previously proposed species are recognized to be closely related with *S. maltophilia* (It might be referred to as '*S. maltophilia* complex' including *Stenotrophomonas pavanii, Stenotrophomonas africana, Pseudomonas geniculata, P. hibisciola*, and *P. beteli*) [16, 24, 25]. *Loci* I, IV, VI, and X were not amplified successfully comparing to reference article [5]. We might postulate that unsuccessful amplification might related to genetically diversity of *S. maltophilia* clinical isolates [3, 4, 15, 16, 23, 24]. In this study, MLVA of *S. maltophilia* isolates was somewhat heterogeneous, but large groups were distinguishable as clade A and B. Although the clustering based on MLVA was not found to link to any characteristics of isolates yet, it is important that the selected SMAGs *loci* used for typing *S. maltophilia* complex. The results of this study suggested that MLVA exhibited higher resolution in using SMAGs *loci* amplification. Amplification locus and the number of amplified *loci* are essential to investigate to obtain sufficiently large clusters without obscuring genotypic links. Epidemiologically, *S. maltophilia* isolates are genetically diverse, and thus clonal dissemination may be rare [16]. These results

Table 3. Sources and antimicrobial activity of *Pseudomonas geniculata* from single hospital

No.	Collection year	Ward	Source	MIC (μg/mL)				
				Tigecycline	Ceftazidime	TMP/SMX	Levofloxacin	TZP
14	2006	ICU	Blood	0.25	16	4:76	1	16:4
58	2008	ICU	Blood	4	16	64:1216	16	16:4
71	2009	ICU	Blood	4	32	16:304	1	16:4
73	2009	GW	Blood	0.5	8	0.25:4.75	1	4:4
102	2008	ICU	Sputum	8	>64	64:1216	16	64:4
106	2010	GW	Pus	2	>64	4:76	2	> 256:4
113	2011	GW	Blood	1	4	32:608	2	8:4
115	2011	GW	Sputum	4	>64	16:304	4	> 256:4
119	2011	ICU	Blood	16	>64	16:304	32	> 256:4
122	2011	ICU	Blood	16	>64	32:608	64	256:4

MIC, minimum inhibitory concentration; TMP/SMX, trimethoprim-sulfamethoxazole; TZP, piperacillin/tazobactam; ICU, intensive care unit; GW, general ward.

are in accordance with earlier studies demonstrating an elevated genetic diversity in *S. maltophilia* isolates even when recovered from the same hospital [6, 23]. Although the isolates examined here exhibited high heterogeneity, homologous isolates (*e.g.*, all *P. geniculata* from one hospital in Seoul) could be recovered from different patients on different ward and at different time points, which may suggest the settle-down of this species in particular hospital and the possibility horizontal transmission among patients (Table 3). These findings further reinforce the postulation that while *S. maltophilia* could be acquired by diverse routes, cross-transmission is also possible. Hence, management of *S. maltophilia* infections would be problematic as the agents may not behave uniformly and transmission may include multiple drug-resistant isolates. These results support efforts directed towards continuous surveillance for antimicrobial drug resistance and epidemiological monitoring, which may act as early warning systems for predicting resistance and preventing outbreaks. The MLVA evaluated in this study provides a fast, portable, relatively low cost genotyping method that can be employed in genotypic linkage or transmission networks comparing to *gyr*B analysis.

Conflicts of Interest

No conflicts of interest.

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