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ORIGINAL ARTICLE

Characterization Integrons and Gene Cassettes in Trimethoprim/Sulfamethoxazole-resistant *Stenotrophomonas maltophilia*

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Background: Integrons in *Stenotrophomonas maltophilia* are the major mechanism for trimethoprim/sulfamethoxazole (TMP/SMX) resistance. Molecular epidemiology of *S. maltophilia* with integrons has not been studied till now, and gene cassettes of the integron in *S. maltophilia* may change with time. **Aim:** Molecular typing and interrelatedness between TMP/SMX-resistant *S. maltophilia* (TSRSM) isolates in a hospital in 2017 were analyzed and gene context of cassettes in integrons was studied. **Methods:** Molecular typing was determined through a multilocus sequence typing (MLST) scheme, while pulsed-field gel electrophoresis (PFGE) was used for relatedness analysis of TSRSM with integrons. Mapping of gene cassettes in the integron was also performed through sequencing. **Results:** From 214 *S. maltophilia* isolates collected in 2017, 34 of them (15.9%) were TSRSM. A total of 20 (58.8%) from 34 TSRSM isolates harboring the class 1 integron were analyzed. The MLST analysis revealed 11 different sequence types, 5 out of which were novel STs (ST 830, ST 833, ST 836, ST 837, and ST 839), suggesting a wide genetic diversity. There were two clones with intrahospital dissemination between different hospital settings, according to PFGE. Mapping of gene cassettes of the integron revealed four novel combinations of multiresistance genes (*aacA4-aadA5*, a*acA7-catB*, *cmlA10-aadA2*, and *aacA4-aphA15-catB3*), indicating the continued evolutionary change of the gene cassettes. **Conclusion:** Evidence of clonal transmission within the hospital and continuous change of multiresistant gene combinations in the cassettes of the integron showed that *S. maltophilia* with resistance integrons may play a role in the spread of antimicrobial resistance.

Key words: *Stenotrophomonas maltophilia,* integrons, resistance

INTRODUCTION

Integrons, which were first described in 1989, are special genetic elements in bacterial genomes that allow for efficient capture and expression of different inserted exogenous genes through a site-specific recombination system.[1,2] In general, all integrons share several essential components: an *intI* gene encodes a site-specific integron integrase that can mediate insertion or excision of incoming genes; *attI*, located adjacent to *intI* recognized by the integrase, is the recombination site which incoming genes may be inserted in; Pc, an integron-associated promoter for integrated gene-expression.[3]

The *intI gene* was defined as *intI1, intI2, intI3,* and *et al.* based on amino acid sequence difference. The integron carrying *intI1*, therefore, is defined as class 1, carrying *intI2* as class 2, carrying *intI3* as class 3, respectively.[3] Class 1, class 2, and class 3 integrons are found in different Gram-positive and Gram-negative bacteria, while class 4 integron is observed only in *Vibrio* species.[4] Integrated genes in the integron bounded by two conserved segments (5′ and 3′ conserved segments: 5′CS and 3′CS) were called gene cassettes. Gene

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cassettes usually consist of a single open reading frame bounded by a cassette-associated recombination site known as *attC* and could transfer to other integrons or to secondary sites in the bacterial genome through site-specific recombination mediated by *intI*.[2] Majority of gene cassettes encode antibiotic resistance genes and form important vehicles for the spread of antibiotic resistance among Gram-negative bacteria.[5,6] More than 100 cassettes carrying known or predicted antibiotic resistance genes have been identified and found in integrons. Those resistance cassettes have been widely known for their role in antimicrobial resistance dissemination.[7]

Among Gram-negative bacteria, class 1 integrons have only been identified in *Stenotrophomonas maltophilia.* Class 1 integrons are reported contributing to resistance to trimethoprim/sulfamethoxazole (TMP/SMX) in *S. maltophilia*.[8] Resistance mechanism may be associated with *sul1* gene in the 3′ conserved segment of the integron, which confers sulfonamide resistance. Resistance conferred by *dfr*-type genes in integrons can further elevate minimum inhibitory concentration (MIC) levels of TMP/SMX.[9,10] High prevalence of class 1 integrons in TMP/SMX-resistant *S. maltophilia* (TSRSM) supports the idea that this class of integron associated with *sul1* gene is the major resistance mechanism for TMP/SMX.[9,11,12] Different resistance gene cassettes in the integron of *S. maltophilia* may evolve with time and may influence resistance phenotypes.[13] Nevertheless, relevant data of incorporated genes in cassettes from *S. maltophilia* with integrons in Taiwan are about 10 years ago, which may have changed significantly.[8,14]

Moreover, with the global emergence of the TMP/SMX-resistant *S. maltophilia,* relevant molecular epidemiology of TSRSM with integrons has not been studied yet.[9] The multilocus sequence typing (MLST) technique appears to be a reliable tool for typing bacterial pathogens among different typing methods.[15] It could provide consistent epidemiological data for evolutionary, phylogenetic, or population genetic studies since the results from different laboratories could be retrieved from easily accessible international databases PubMLST (https://pubmlst.org/).

Therefore, the aim of the present study is to map gene cassettes in TSRSM with integrons to investigate evolutionary resistance gene changes. Besides, the molecular epidemiology and interrelatedness of TSRSM with integrons were also studied.

MATERIALS AND METHODS

Bacterial strains

S. maltophilia isolates were consecutively obtained as part of routine hospital care procedures from a variety of specimens of admitted patients at the tri-service general hospital in 2017. Only one isolate was obtained from each patient. The VITEK 2 automatic system (bioMérieux Inc., Marcy-l'Etoile, Rhône, France) was used to identify *S. maltophilia* isolates. The identification protocol was according to the manufacturer's instructions as described before.[16] All identified *S. maltophilia* were stored at −80°C in GermBank stocks (CMPTM Culture Media, New Taipei City, Taiwan) until processing. For the aims of the study, *S. maltophilia* isolates were tested antimicrobial susceptibility and integrons existence. Only TSRSM isolates with integrons confirmed by polymerase chain reaction (PCR) were included for further investigation.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for TMP/SMX, amikacin, gentamicin, levofloxacin, ciprofloxacin, tigecycline, ceftazidime, and colistin was performed using the VITEK 2 automatic system. Susceptibility to minocycline and cefiderocol was tested using the disk diffusion method. Susceptibility interpretation for TMP/SMX was based on 2021 CLSI criteria. Isolates resistant to TMP/SMX were defined as the minimal inhibitory concentration MIC to TMP/SMX ≥4/76 μg/mL MIC levels of identified *S. maltophilia* isolates for amikacin, gentamicin, levofloxacin, ciprofloxacin, tigecycline, ceftazidime, and colistin was also measured using the VITEK 2 automatic system for *in vitro* activity comparisons. Susceptibility interpretation for amikacin, gentamicin, ciprofloxacin, and colistin was performed according to the 2021 CLSI criteria for *Pseudomonas aeruginosa*. Tigecycline breakpoints were established as per the 2021 European Committee on Antimicrobial Susceptibility Testing for *Enterobacterales*.

Multilocus sequence typing and interrelatedness analysis

The MLST was performed for typing of *S. maltophilia* isolates as described earlier with some modifications.[17] Initially, *S. maltophilia* isolates from GermBank stocks (CMPTM Culture Media, New Taipei City, Taiwan) were subcultured with aeration in Luria-Bertani plate at 37°C overnight. Next, total DNA extraction for PCR reaction was performed using DNAzol reagent (Invitrogen, Waltham, Massachusetts, USA) according to the manufacturer's protocol. Seven housekeeping genes, *recA, guaA gapA*, *nuoD*, *ppsA*, *MutM*, and *atpD,* of *S. maltophilia* isolates which have been used for the MLST scheme to study the population structure, were selected. Seven pairs of primer sequences targeting the conservative region of 7 housekeeping genes described before used for further PCR reactions were shown in Table 1.[17] Each PCR reaction used to amplify target fragments was performed as follows: initial denaturation at 94°C (15 min) followed by 35 cycles of denaturation at 94°C (30 s), annealing at 62°C (90 s) for

Table 1: Primers used in the study

Primers	Target	Sequence (5′–3′)	Source
AtpD-F	*AtpD*	ATGAGTCAGGGCAAGATCGTTC	[17]
AtpD-R		TCCTGCAGGACGCCCATTTC	
GapA-F	*GapA*	TGGCAATCAAGGTTGGTATCAAC	[17]
GapA-R		TTCGCTCTGTGCCTTCACTTC	
GuaA-F	*GuaA*	AACGAAGAAAAGCGCTGGTA	[17]
GuaA-R		ACGGATGGCGGTAGACCAT	
MutM-F	*MutM*	AACTGCCCGAAGTCGAAAC	[17]
MutM-R		GAGGATCTCCTTCACCGCATC	
NuoD-F	*NuoD*	TTCGCAACTACACCATGAAC	[17]
NuoD-R		CAGCGCGACTCCTTGTACTT	
PpsA-F	*PpsA*	CAAGGCGATCCGCATGGTGTATTC	[17]
PpsA-R		CCTTCGTAGATGAA (A/G) CCGGT (A/G) TC	
RecA-F	*RecA*	ATGGACGAGAACAAGAAGCGC	[17]
RecA-R		CCTGCAGGCCCATCGCC	
5′ CS	Gene cassette in class 1 integron	GGCATCCAAGCAGCAAG	[18]
3′CS	Gene cassette in class 1 integron	AAGCAGACTTGACCTGA	[18]
Int1-F	Class 1 integrase	CAGTGGACATAAGCCTGTTC	[18]
Int1-R		CCCGAGGCATAGACTGTA	[18]
Int2-F	Class 2 integrase	GTAGCAAACGAGTGACGAAATG	[18]
Int2-R		CACGGATATGCGACAAAAAGGT	[18]
Int3-F	Class 3 integrase	GCCTCCGGCAGCGACTTTCAG	[18]
Int3-R		ACGGATCTGCCAAACCTGACT	[18]
Sul1-R	-	GCCGATCGCGTGAAGTTCCG	[18]

atpD, 62°C for *gapA*, 56°C for *guaA*, 56°C for *mutM*, 48°C for *nuoD*, 60°C for *ppsA*, and 60°C for *recA*, and extension step at 72°C (1 min 30 s), with a final extension step at 72°C (4 min). The PCR amplicons then were sequenced by Genomics Company (New Taipei City, Taiwan). The sequenced data of each housekeeping gene fragment was then submitted to the MLST database of *S. maltophilia* (http://pubmlst.org/smaltophilia/) for allele determination and then constituted allele profiles of each isolate. Specific STs of *S. maltophilia* would finally be designated based on different allele profiles. Phylogenetic analysis was performed based on the concatenated data for all seven housekeeping genes from *S. maltophilia* isolates, and unrooted neighbor joining (NJ) trees were generated using the Kimura two-parameter model. Dendrograms were generated by NJ method with arithmetic means following bootstrap analysis (1000 replications) using Molecular Evolutionary Genetics Analysis software.[19] For TSRSM of identical STs, further pulsed-field gel electrophoresis (PFGE) was performed to analyze relatedness between strains. PFGE for TSRSM was performed according to a previously reported modified protocol by CS Shueh *et al*.[20] Briefly, the overnight-grown bacterial cells were embedded in 2% low-melting-point agarose plugs. These cells were then treated with proteinase K, and the genomic DNA in the plugs was digested with restriction endonuclease XbaI. The resultant fragments were resolved by PFGE in a CHEF electrophoresis system (Bio-Rad) 24 h at 14°C and 5–35 s for linear ramping at 6 V cm^{-1}). The similarity index was calculated with dice coefficient and dendrogram constructed using the UPGMA algorithm using GelCompar II software (Applied Maths, Belgium).

Polymerase chain reaction amplification and sequencing of class 1, 2, and 3 integrons

S. maltophilia isolates with TMP/SMX resistance were screened for the presence of different *intI* genes to determine the existence of a specific class of integron (class 1, class 2, and class 3 integrons were defined as carrying the *intI1*, *intI2,* and *intI3* gene, respectively) by PCR method as described with some modification.[18] Primer pairs of different *intI* genes used for PCR amplification and sequencing are shown in Table 1. PCR reaction used to amplify target fragments was

performed as follows: initial denaturation at 95°C for 15 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 48°C (90 s) for *intI1*, 55°C for *intI2*, 52°C for *intI3*, extension at 72°C for 1 min and ended with a final extension at 72°C for 5 min. Sequencing of purified PCR products was carried out by Genomics (New Taipei City, Taiwan). Successful sequenced segments were submitted to the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov) and aligned with the already annotated deposited integrase genes using the basic local alignment search tool (BLAST) to confirm accuracy. The content and order of the antibiotic resistance genes inserted between 5′ CS and 3′ CS using primers were assessed as previously described.[8] Due to variable 3′ CS of class 1 integron, another set of primer pairs, 5′CS (5′-GGCATCCA AGCAGCAAG-3′) and *sul1*-R (5′-GCCGATCGCGT GAAGTTCCG-3′), was used to extend gene segment, including partial sequencing of *sul1* genes located downstream of the 3′ CS of class 1 integron, to find possible existence of gene cassettes.[21] Amplified gene cassettes were sequenced and submitted to the NCBI website and aligned with already deposited gene cassettes sequences from bacteria (www.ncbi.nlm.nih.gov). BLAST computer algorithm was used to determine the content and order of the antibiotic resistance genes inserted in the integron.

Ethical approval

This study was approved by the Institutional Review Board of Tri-Service General Hospital (Approval number: 1-103-05-154). The patient consent was waived by the IRB.

RESULTS

Characteristics and antimicrobial susceptibility of trimethoprim/sulfamethoxazole resistant *Stenotrophomonas maltophilia* isolates with integrons

In this study, 219 clinical *S. maltophilia* isolates were collected in 2017. One hundred eighty-five isolates (84.4%) were from sputum specimens, 9 from wound exudates (4.1%), 2 from urine (0.9%), 21 from blood (9.6%), and 2 from ascites fluid (1.0%). Among the 219 identified *S. maltophilia* isolates, 34 were TSRSM with 15.5% prevalence.

Out of the 34 TSRSM isolates, 20 of them (58.8%) had class 1 integron based on the presence of class 1 integrase gene. No TSRSM isolates with class 2 or class 3 integrons were identified. Characteristics of TSRSM isolates with class 1 integrons are shown in Table 2. The major clinical specimens from which TSRSM with integrons was isolated were from the respiratory tract. Eleven out of 20 TSRSM (55%) with integrons were from the intensive care unit (ICU) samples.

The results of antimicrobial susceptibility and MICs against antibiotics of TSRSM with integrons are depicted in Table 3. TSRSM exhibited the highest susceptibility of 95% and 100% for minocycline and cefiderocol, respectively. Nonsusceptibility rates of TSRSM with integrons to other potential *in vitro* active antibiotics, including ceftazidime, levofloxacin, ciprofloxacin, colistin, tigecycline, amikacin, and gentamicin were all higher than 50%.

Molecular epidemiology and phylogenetic analysis of trimethoprim/sulfamethoxazole resistant *Stenotrophomonas maltophilia* isolates with integrons

Among 20 TSRSM with class 1 integrons, 14 of them (70.0%) belonged to a known ST, and ST 27 and ST 791 were the major ones (both $n = 5$). Six isolates belonged to new STs, namely ST 830 ($n = 1$), ST 833 ($n = 1$), ST 836 ($n = 1$), ST 837 ($n = 2$), and ST 839 ($n = 1$). Those isolates

Table 2: Characteristics of trimethoprim/sulfamethoxazole-resistant *Stenotrophomonas maltophilia*, with integrons

Strain	Isolation date	Specimen	Place isolation	STs from MLST
SM468	January 03, 2017	Sputum	Cardiology ward	27
SM469	January 03, 2017	Sputum	Cardiology ward	27
SM470	January 04, 2017	Sputum	Nephrology ward	27
SM930	February 01, 2017	Blood	Neurology ward	27
SM582	February 02, 2017	Blood	Cardiology ICU	27
SM507	February 03, 2017	Sputum	Medical ICU	31
SM591	February 06, 2017	Sputum	Cardiology ICU	208
SM1000	February 08, 2017	Sputum	Surgical ICU	365
SM619	February 09, 2017	Sputum	Medical ICU	839
SM707	February 09, 2017	Sputum	Infection ward	365
SM543	February 10, 2017	Sputum	Surgical ICU	791
SM574	February 10, 2017	Sputum	Surgical ICU	791
SM646	February 13, 2017	Sputum	Burn center	791
SM716	February 17, 2017	Sputum	Oncology ward	791
SM1565	February 24, 2017	Urine	Chest medicine ward	791
SM522	March 07, 2017	Sputum	Neurology ICU	830
SM993	March 09, 2017	Sputum	Neurology ward	833
SM530	April 30, 2017	Sputum	Infection ICU	836
SM577	May 16, 2017	Wound	Infection ICU	837
SM578	May 18, 2017	Sputum	Infection ICU	837

TSRSM=Trimethoprim/sulfamethoxazole-resistant *Stenotrophomonas maltophilia*; STs=Sequence types; MLST=Multilocus sequence typing; ICU=Intensive care unit

with novel STs were all from ICU settings except isolate SM 522, which was isolated from patients in the neurology ward. Nevertheless, the patient which SM 522 cultured from still had a history of recent ICU admission within 2 weeks before isolation. The phylogenic tree of 20 isolates belonging to different STs with no major clustering of strains is shown in Figure 1. Moreover, isolates with novel STs did not form a cluster indicating that those isolates evolved independently rather than from a common origin. Further PFGE analysis for available isolates of ST27 (SM 469, SM470, SM930, and SM582) and those of ST 791(SM 574, SM 716, SM1565) revealed similar PFGE pattern between isolates with identical STs, indicating possible cross-transmission among the admitted patients [Figure 2a and b].

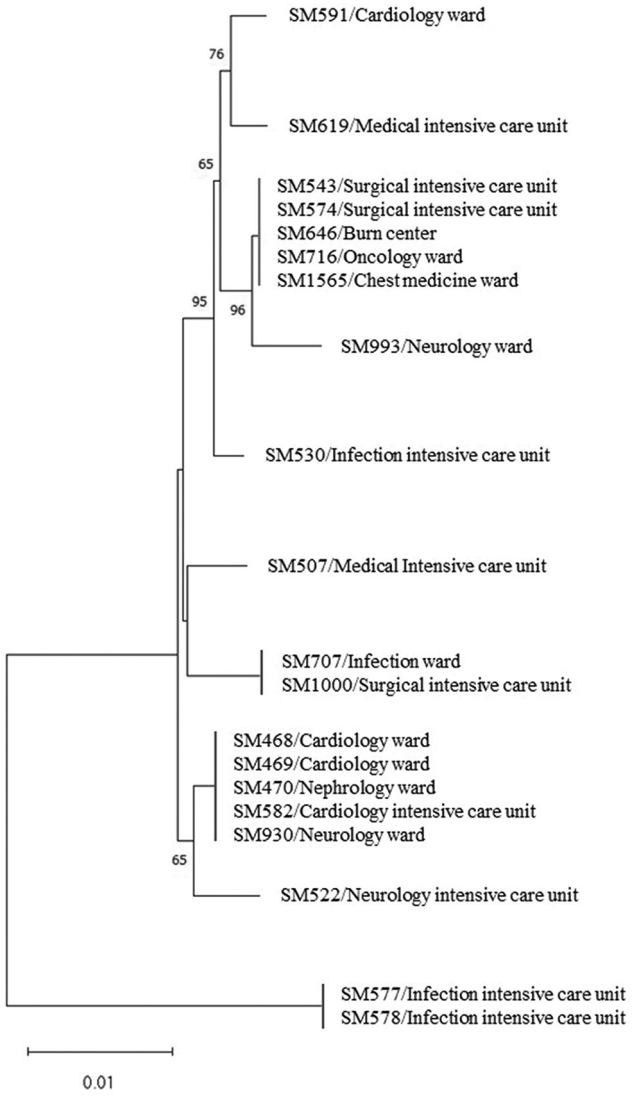

Figure 1: Phylogenetic tree using neighbor-joining method based on the concatenated data of the seven housekeeping genes of the 20 trimethoprim-sulfamethoxazole-resistant *Stenotrophomonas maltophilia* isolates with integrons. The scale bar indicates the number of nucleotide substitutions per site

Gene cassettes mapping

Among 20 TSRSM isolates with integrons, gene cassettes of varied sizes showed mono- or multiantibiotic resistance genes from 0.5 kb to 1.5 kb, as determined through primers 5′-CS and 3′-CS. Four different cassette rearrangements were observed [Table 2]: *SMR* conferring resistance to disinfectants; *aacA4*, *aadA2,* and *aacA4-aadA5* conferring resistance to aminoglycosides. Gene cassettes from five integrase 1-positive *S. maltophilia* were not obtained using primer 5′-CS and 3′-CS. The primers 5′-CS and *Sul1*-R were used to further examine the genetic context of the class 1 integrons in such isolates. All five *S. maltophilia* isolates exhibited loss of 3′-conserved segment. Of the five *S. maltophilia* isolates without 3′-CS, three had different cassette rearrangements (*aacA7-catB*, *cmlA10-aadA2*, *aacA4-aphA15-catB3*) and one isolate SM 619 had an empty gene cassette. Resistance genes *aacA7, aadA2, aacA4,* and *aphA15* conferred resistance to aminoglycosides, while *catB*, *cmlA10,* and *catB3* conferred resistance to chloramphenicol. Four different cassette rearrangements *aacA4-aadA5* in SM 1565, *aacA7-catB* in SM577 and SM578, *cmlA10-aadA2* in SM591, and *aacA4-aphA15-catB3* in SM 619 were firstly reported in *S. maltophilia* isolates. Among those isolates with novel resistance gene arrays in cassettes, three (SM619, SM577, and SM 578) had novel STs and were from ICU

Table 3: Susceptibility of 20 trimethoprim/sulfamethoxazole-resistant *Stenotrophomonas maltophilia* isolates with integrons to different antibiotics

Antibiotics	MIC (mg/L), range	Number of indicated susceptibility category		
		S	*I*	*R*
Ceftazidime	≤1–≥64	11 (55)	2 (10)	7 (35)
Levofloxacin	1–≥8	2 (10)	8 (40)	10 (50)
Ciprofloxacin[b]	1–≥4	0	1 (5)	19 (95)
Tigecycline[a]	≤0.5–≥8	2 (10)		18 (90)
Colistin[b]	≤0.5–≥16	-	10 (50)	10 (50)
Amikacin	8–≥64	5 (25)	3 (15)	12 (60)
Gentamicin	4–≥16	2 (10)	4 (20)	14 (70)
Cefiderocol[c]	-	20 (100)		
Minocycline[c]	-	19 (95)	1 (5)	0

[a]Tigecycline breakpoints established by the 2021 EUCAST for *Enterobacteriaceae*; [b]Breakpoints established by the 2021 CLSI for *Pseudomonas aeruginosa*; [c]Results from disk diffusion test. TSRSM=Trimethoprim/sulfamethoxazole resistant *Stenotrophomonas maltophilia*; MIC=Minimum inhibitory concentration; EUCAST=European Committee on Antimicrobial Susceptibility Testing; CLSI=Clinical and laboratory standards institute

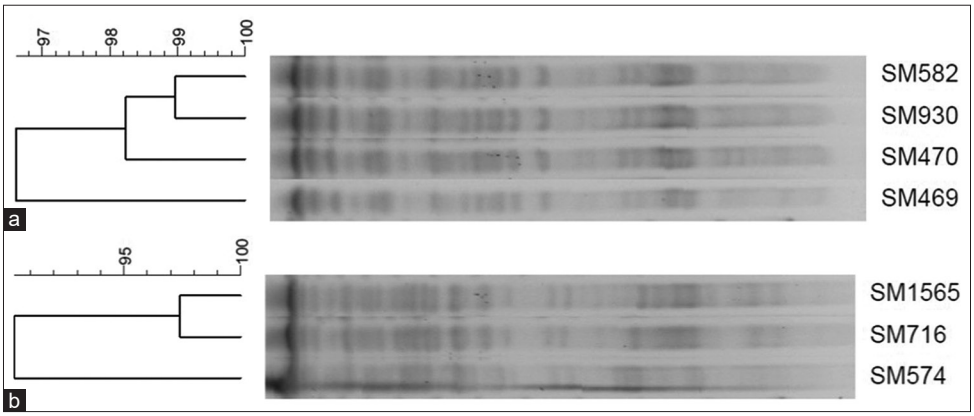

Figure 2: PFGE profiles of clinical *Stenotrophomonas maltophilia* isolates of (a) ST27 and (b) ST791. Similar banding patterns were observed between isolates of the same ST. The scale indicates percent similarity. PFGE = Pulsed-field gel electrophoresis

settings. Moreover, four isolates (SM577, SM578, SM591, and SM619) showed loss of 3′-conserved segment.

DISCUSSION

In the present study, we reported the antimicrobial susceptibility, molecular epidemiology, and mapping of gene cassettes in TSRSM isolates with integrons from a single hospital. Multiple drug resistance phenotypes were observed among TSRSM isolates with integrons, with only cefiderocol and minocycline showing good *in vitro* activity. High degree of genetic diversity among TSRSM isolates with integrons based on MLST. According to further PFGE for isolates with the same ST, there is clonal dissemination among patients from different wards in the hospital. Several resistance gene arrays were firstly observed indicating evolutionary change of class 1 integron gene cassette in *S. maltophilia* over the years is ongoing.

The antimicrobial susceptibility for potential *in vitro* antibiotics revealed that *S. maltophilia* with integrons had high nonsusceptibility rates except for minocycline and cefiderocol, which is consistent with previous reports.[22,23] The clinical outcomes of patients administered with either minocycline or cefidercol are limited, though these had promising *in vitro* activity.[24,25] This calls for more studies to validate the effectiveness of minocycline and cefiderocol in clinical practice.

Although the 20 TSRSM isolates with integrons examined were isolated from the same hospital, there was a high degree of genetic diversity between isolates, according to MLST. Six isolates belonged to novel STs, and the profiles of STs tested differed from those found in Europe and Japan.[17,26] Although limited isolates were observed, the data suggest that there is a weak genetic linkage between *S. maltophilia* strains in different countries. Moreover, integrons with the same gene cassettes profiles could be observed in *S. maltophilia* of different STs in the present study. The results indicated that integrons might play a key role in the dissemination and spread of resistance genes among *S. maltophilia.* PFGE findings from isolates of the same STs provided evidence of intrahospital dissemination. Similar pulsotypes between tested isolates from different wards implied the presence of clonal dissemination among the patients from different wards in the hospital. However, *S. maltophilia* isolates were not preserved, and clinical information of infected patients was also not recorded. Hence, further investigation to find the possible source of dissemination cannot be conducted. A high frequency of class 1 integrons was observed among TSRSM isolates (58.8%), which corroborates well with previous reports.[8,11] TMP/SMX resistance may be mediated by *sul* genes located at the 3′-CS of the class 1 integrons.[9] Notably, there were some TSRSM isolates without integrons, suggesting other possible resistance mechanisms demonstrated by earlier workers.[10,27] Further investigation for such isolates is underway. Typically, a class 1 integron structure contains *IntI1* on the 5′-CS and qacEΔ1-sul1 on the 3′-CS.[3] In this study, five isolates were found to contain qacEΔ1-sul1 genes without 3′-CS, which is consistent with previous findings showing different structures of the 3′conserved region of class 1 integrons.[21] Furthermore, among the *intI1*-positive isolates, a total of seven kinds of resistance gene cassettes were detected [Table 4]. One isolate had no cassettes (empty class 1 integron). The most common gene cassettes located in the class 1 integrons of *S. maltophilia* isolates were *smr* and *aacA4*. Other resistance genes detected in cassettes included *smr, aacA4, aadA2, aacA4, aadA5, aacA7, aphA15*, *cmlA,* and *catB,* which have already been reported in *S. maltophilia*.[23] Although no new resistance genes were found, several resistance gene arrays were observed for the first time, including *aacA4-aadA5, aacA7-catB, cmlA10-aadA2,* and *aacA4-aphA15-catB3.* Notably, *dfr*-type genes associated

Table 4: Gene cassette arrays of class 1 integrons among *Stenotrophomonas maltophilia* isolates

Bacterial strain	Amplicon size (bp)	Gene cassette array	Number of isolates (%)
SM469, SM470, SM507, SM522, SM582, SM646, SM930	500	*SMR*	7
SM468, SM543, SM574, SM716, SM993	750	*aacA4*	5
SM707, SM1000	1000	*aadA2*	2
SM1565	1500	*aacA4-aadA5*	1
SM530	-	empty	1
SM577, SM578	-	*aacA7- catB*	2
SM591	-	*cmlA10-aadA2*	1
SM619	-	*aacA4-aphA15-catB3*	1

with TMP resistance often reported in *S. maltophilia* and other Gram-negative bacteria with class 1 integrons were not identified in the present study.[12,28] Therefore, we could not evaluate the influence of *dfr*-type genes on the MIC of TMP/SMX in TSRSM isolates. The data obtained in this study indicate the ongoing evolutionary change of class 1 integron gene cassette in *S. maltophilia* over the years, and continued surveillance to evaluate the influence on resistance phenotype is thus warranted.

CONCLUSION

We observed diversity in STs among TSRSM isolates with integrons and a small-scale intra-hospital clonal dissemination. Mapping of gene cassettes suggested that integrons with continued embedded gene change could successfully disseminate between *S. maltophilia* isolates and may promote antibiotic resistance spread since mobile gene cassettes of integrons may move to other species of Gram-negative bacteria. Hence, continued monitoring of the ongoing evolutionary change of class 1 integron gene cassette in *S. maltophilia* and its influence on resistance phenotype and other Gram-negative bacteria are needed.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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