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Occurrence and Characteristics of Class 1 and 2 Integrons in *Pseudomonas aeruginosa* Isolates from Patients in Southern China▽

Zhenbo Xu,[1,2] Lin Li,[1] Mark E. Shirtliff,[2] M. J. Alam,[3] Shinji Yamasaki,[1,4] and Lei Shi[1,4]*

College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, China[1]; Department of Microbial Pathogenesis, Dental School, University of Maryland—Baltimore, 7th Floor South, 650 W. Baltimore Street, Baltimore, Maryland 21201[2]; College of Agriculture & Life Sciences, Texas A & M University, College Station, Texas 77843[3]; and Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan[4]

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Class 1 and 2 integrons were detected in 45.8% (54/118) and 19.5% (23/118) of our tested *Pseudomonas aeruginosa* isolates, respectively. Three strains were positive for both the integrons. This is the first report of class 2 integrons in *P. aeruginosa* and also of isolates carrying class 1 and 2 integrons simultaneously.

Pseudomonas aeruginosa remains one of the most important pathogens in the nosocomial setting (14), and it not only is naturally resistant to many antimicrobial agents but also has the distinctive capacity via multiple mechanisms to become resistant to virtually all the antibiotics available commercially (9, 38). A genetic element, the integron, is potentially a major agent in the dissemination of multidrug resistance among gram-negative bacteria, especially in *Pseudomonas* (16). Gene cassettes, present in the variable region of integrons, are discrete mobile units comprising a gene, usually an antibiotic resistance gene, and a recombination site that is recognized by an integrase. The class 1 integron has been identified as a primary source of resistance genes within gram-negative and -positive bacteria (6, 20, 33, 36, 40, 41, 42), and the class 2 integron has been seen in *Acinetobacter* sp. isolates throughout the world (28). However, class 2 integrons in *P. aeruginosa* strains had not yet been investigated. In this study, 118 imipenem-resistant *P. aeruginosa* isolates were chosen for the investigation of class 1 and 2 integrons because of the relatively high integron-positive rate in imipenem-resistant isolates.

From 2001 to 2005, a total of 118 consecutive nonduplicated *P. aeruginosa* isolates which were intermediate or resistant (nonsusceptible) to imipenem (IMP; MIC > 8 μg/ml) were isolated from the First Affiliated Hospital of Jinan University, an 850-bed tertiary-level teaching hospital in Guangzhou, China. Identification of isolates to the species level and antimicrobial susceptibility testing were performed with the Vitek system (bioMerieux Vitek Systems Inc., Hazelwood, MO). The quality control strain used was *P. aeruginosa* ATCC 27853. Template DNA used for PCR was prepared as described previously (16). Detection and characterization of class 1 and 2 integrons were performed as described previously (35, 41). PCR products of the variable region were further characterized by restriction fragment length polymorphism (RFLP), and at least two different restriction endonucleases were chosen for each RFLP assay, and the DNA sequence for at least one of the variable region amplification products belonging to each of the individual RFLP patterns was determined as described previously (35). Seventy-four integron-positive *P. aeruginosa* isolates were subjected to genotyping analysis by randomly amplified polymorphic DNA PCR (RAPD-PCR) as described previously (35).

The multidrug resistance (defined as resistance to six or more antibiotics) rates of integron-positive and -negative strains were 93.2% and 18.2%, respectively (Table 1). Class 1 integron was detected in 54 isolates, and 51 strains carried the 3′ conserved region of *qacEΔ1-sul1*. Seven different sizes of variable region were found, with fragments with lengths ranging between 879 bp and 2,655 bp (Table 2). The array of the *aacA4-catB3-dfrA1* noncoding gene cassette has been reported previously (16). The defective class 1 integron with a *sul3* gene, which was identical with that seen in *Salmonella enterica* serovar Typhimurium (AY047357), had never been reported to be seen in isolates of *P. aeruginosa*. Class 2 integrons were found in 23 *P. aeruginosa* isolates, and all strains harbored the same array of three cassettes, *dfrA1-sat1-aadA1*, which was identical to that found in Tn*7*. Three strains had both class 1 and 2 integrase genes. No class 3 integrase gene was detected in any of the isolates examined. RAPD-PCR analysis divided 74 integron-positive *P. aeruginosa* strains into eight different groups with different RAPD patterns (genotypes A to H) (Fig. 1). Fifty-one class 1 integron-positive strains and 3 class 1 and 2 integron-positive strains were of types A, B, C, F, G, and H, and 20 class 2 integron-positive strains were of types D and E (Table 2).

Integrons have been identified as a primary source of resistance genes and were suspected to serve as reservoirs of

* Corresponding author. Mailing address: College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, People's Republic of China. Phone and fax: 86-20-87112734. E-mail: leishi88@hotmail.com.

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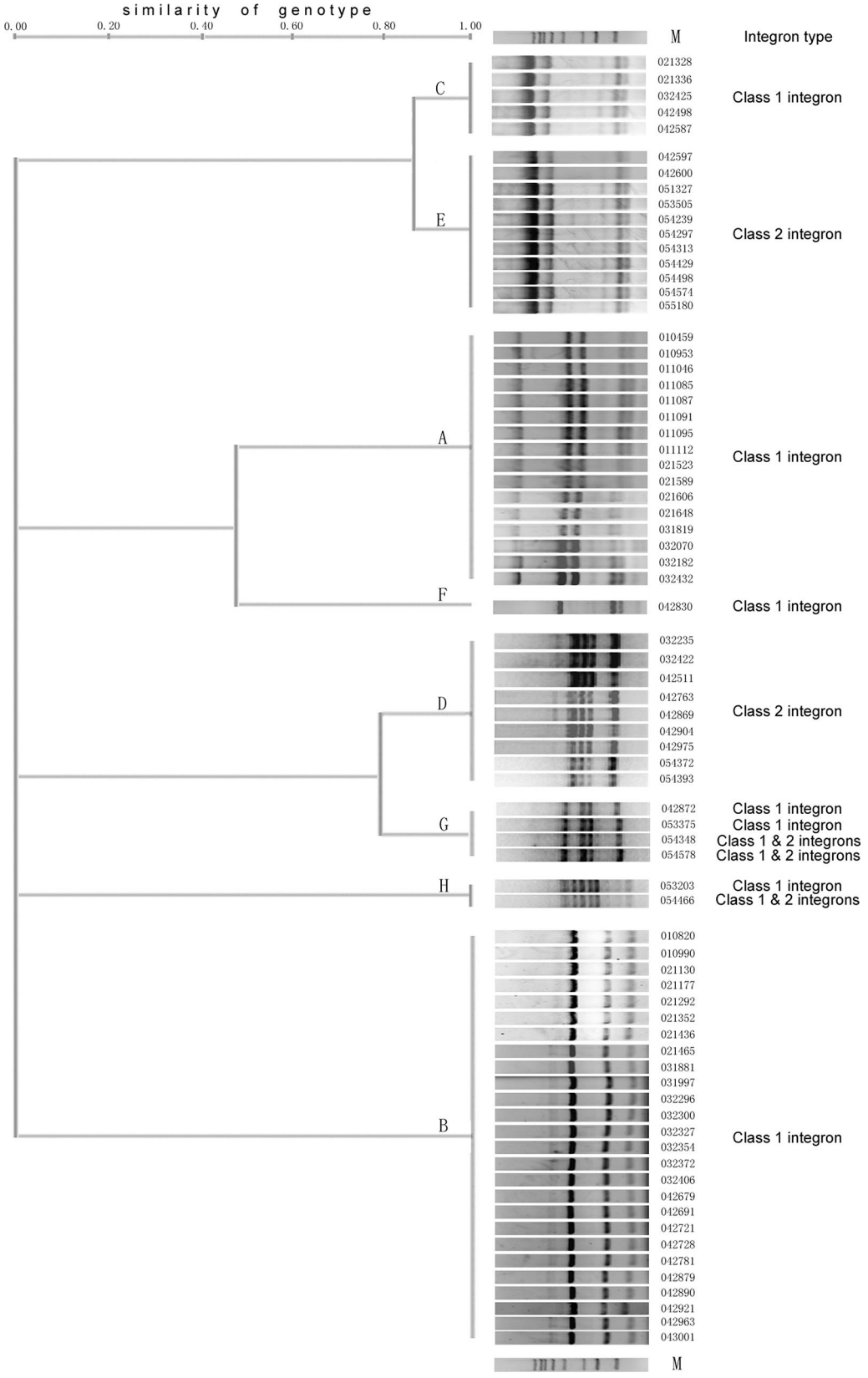

FIG. 1. RAPD-PCR patterns of 74 integron-positive *Pseudomonas aeruginosa* isolates.

TABLE 1. Association between antibiotic susceptibility profile and integrons in 118 *Pseudomonas aeruginosa* isolates

Antibiotic[a]	% (no.) of isolates								
	Total (n = 118)			Integron-positive isolates (n = 74)			Integron-negative isolates (n = 44)		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
AMK	33.1 (39)	13.6 (16)	53.3 (63)	44.6 (33)	18.9 (14)	37.5 (27)	13.6 (6)	4.5 (2)	81.9 (36)
ATM	41.5 (49)	14.4 (17)	44.1 (52)	55.4 (41)	14.9 (11)	29.7 (22)	18.2 (8)	13.6 (6)	68.2 (30)
CAZ	37.3 (44)	7.6 (9)	55.1 (65)	50.0 (37)	5.4 (4)	44.6 (33)	15.9 (7)	11.4 (5)	72.7 (32)
CIP	70.3 (83)	11.0 (13)	18.6 (22)	79.7 (59)	12.2 (9)	8.1 (6)	54.5 (24)	9.1 (4)	36.4 (16)
CRO	42.4 (50)	6.8 (8)	50.8 (60)	50.0 (37)	6.8 (5)	43.2 (32)	29.6 (13)	6.8 (3)	63.6 (28)
GEN	63.6 (75)	11.0 (13)	25.4 (30)	85.1 (63)	14.9 (11)	0 (0)	27.3 (12)	4.5 (2)	68.2 (30)
LVX	61.0 (72)	19.5 (23)	19.5 (23)	70.3 (52)	21.6 (16)	8.1 (6)	45.5 (20)	15.9 (7)	38.6 (17)
PIP	55.1 (65)	5.9 (7)	39.0 (46)	70.3 (52)	8.1 (6)	21.6 (16)	29.6 (13)	2.2 (1)	68.2 (30)
SXT	70.3 (83)	16.9 (20)	12.7 (15)	79.7 (59)	18.9 (14)	1.4 (1)	54.5 (24)	13.6 (6)	31.9 (14)
TCC	45.8 (54)	10.2 (12)	44.1 (52)	55.4 (41)	16.2 (12)	28.4 (21)	29.6 (13)	0 (0)	70.5 (31)
TET	78.0 (92)	7.6 (9)	14.4 (17)	83.7 (62)	9.5 (7)	6.8 (5)	68.2 (30)	4.5 (2)	27.3 (12)
TOB	55.1 (65)	21.2 (25)	23.7 (28)	75.7 (56)	24.3 (18)	0 (0)	20.5 (9)	15.9 (7)	63.6 (28)

[a] AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CRO, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; LVX, levofloxacin; PIP, piperacillin; TET, tetracycline; TCC, ticarcillin-clavulanic acid; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole.

antimicrobial resistance genes within microbial populations (34), and integron-mediated resistance to antibiotics in clinical isolates of *P. aeruginosa* has been reported (11, 16, 18, 24, 26). However, all of these studies were concerned with class 1 integrons, with no exception. Class 2 integrons were most frequently associated with members of the family *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella enterica*, and also are commonly found in *Acinetobacter baumannii* and *Burkholderia cepacia* (1, 3, 4, 19, 25, 27, 37). However, class 2 integrons in *P. aeruginosa* had never been reported. In this study, we detected 51 class 1 integron-positive strains, 20 class 2 integron-positive strains, and 3 class 1 and 2 integron-positive strains from total of 118 strains. This is the first report, to our knowledge, of class 2 integrons with *dfrA1-sat1-aadA1* in *P. aeruginosa*. Furthermore, it is also the first time clinical *P. aeruginosa* isolates carrying class 1 and 2 integrons simultaneously have been identified.

Class 1 integrons were commonly found in the tested *P. aeruginosa* isolates (45.8%, 54/118), but the class 1 integron-positive rates had been decreasing during the 5-year study period, with rates of 66.6% (10/15) in 2001, 60.0% (12/20) in 2002, 52.0% (13/25) in 2003, 40.0% (14/35) in 2004, and 21.7% (5/23) in 2005. Class 2 integron appeared in 2003, with the class 2 integron-positive rates rising for the next three years, with rates of 8.0% (2/25) in 2003, 20.0% (7/35) in 2004, and 60.8% (14/23) in 2005, indicating that class 2 integron had been prevalent in recent years. The rate of integron-positive isolates had changed in a small scale, with rates of 66.6% in 2001, 60% in 2002 to 2004, and 69.5% in 2005, while the proportion of class 1 integrons had decreased more than 45% and the occurrence of class 2 integron began in 2003. The class 2 integron-positive rate increased to >60% in 2005, suggesting that class 2 integrons were increasing and suggesting and the possibility of this class replacing class 1 integron in recent years. The evolutionary success of an integron was determined by two important factors: the resistance cassettes it carries and the host range of the plasmid on which it occurs (13). The two most frequently detected resistance genes in 74 integron-positive isolates were of the *aadA* and *dfrA* families, with rates of 79.7% (59/74) and 64.9% (48/74), respectively. Since the two cassettes, *dfrA1* and *aadA1*, have been observed in all class 2 integron-positive isolates, it is reasonable to presume the transferring of cassettes among different integrons (13). So whether class 2 integrons have more fitness and better survival ability than class 1 integrons under selective pressure and whether some cassettes appear to have been transferred among integron classes require further investigation.

In conclusion, this study showed the occurrence and characteristics of class 1 and 2 integrons in clinical *P. aeruginosa*. Nevertheless, further studies need to be conducted to investigate the cause of the appearance and prevalence of class 2 integrons in *P. aeruginosa* in recent years. The findings will help to develop control strategies for infections in hospitals.

Nucleotide sequence accession number. The nucleotide sequence accession number of the defective class 1 integron with *sul3* gene in GenBank is AB281182.

TABLE 2. Phenotypic and genotypic characteristics of 74 integron-positive *Pseudomonas aeruginosa* isolates

Strain	Yr of isolation	Source	Age (yr), sex[a]	Genetic material in isolate with[b]:					RAPD pattern	Resistance profile[c]
				Class 1 integrons			Class 2 integrons			
				intI1	3′ conserved sequence	Gene cassette	*intI2*	Gene cassette		
010459	2001	Blood	67, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
010820	2001	Sputum	79, F	+	+	*aacA4-cmlA1*	−	−	B	AzCaCeCiGLPTTcToTs
010953	2001	Blood	80, M	+	+	*dfrA17-aadA5*	−	−	A	ACiGLTToTs
010990	2001	Sputum	80, M	+	−	*aacA4-cmlA1*	−	−	B	AzCaCeCiGLPTTcToTs
011046	2001	Sputum	51, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
011085	2001	Sputum	73, F	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
011087	2001	Sputum	45, F	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
011091	2001	Blood	45, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
011095	2001	Sputum	54, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
011112	2001	Stool	65, F	+	+	*dfrA17-aadA5*	−	−	A	ACiGLTToTs
021130	2002	Stool	54, M	+	+	*aacA4-cmlA1*	−	−	B	AzCaCeCiGLPTTcToTs
021177	2002	Sputum	30, M	+	+	*aacA4-cmlA1*	−	−	B	AzCaCeCiGLPTTcToTs
021292	2002	Blood	72, F	+	+	*aacA4-cmlA1*	−	−	B	AzCaCeCiGLPTTcToTs
021328	2002	Sputum	81, F	+	+	*aacA4-cmlA1*	−	−	C	ACeGPToTs
021336	2002	Sputum	69, M	+	+	*aacA4-cmlA1*	−	−	C	ACeGPToTs
021352	2002	Sputum	69, F	+	+	*aacA4-cmlA1*	−	−	B	AzCaCeCiGLPTTcToTs
021436	2002	Pus	45, M	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
021465	2002	Sputum	30, F	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
021523	2002	Sputum	65, F	+	−	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
021589	2002	Stool	25, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
021606	2002	Sputum	77, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
021648	2002	Blood	42, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
031819	2003	Sputum	75, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
031881	2003	Sputum	83, F	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
031997	2003	Pus	21, F	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032070	2003	Sputum	80, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
032182	2003	Stool	26, F	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
032235	2003	Sputum	73, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
032296	2003	Blood	72, M	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032300	2003	Sputum	78, F	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032327	2003	Stool	22, M	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032354	2003	Sputum	72, F	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032372	2003	Blood	76, M	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032406	2003	Stool	70, F	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
032422	2003	Sputum	72, M	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
032425	2003	Blood	72, F	+	−	*aadA2*	−	−	C	ACeGPToTs
032432	2003	Sputum	47, M	+	+	*dfrA12-orfF-aadA2*	−	−	A	ACiGLTToTs
042498	2004	Blood	85, M	+	+	*aadA2*	−	−	C	ACeGPToTs
042511	2004	Sputum	67, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
042587	2004	Blood	24, M	+	+	*aadA2*	−	−	C	ACeGPToTs
042597	2004	Sputum	65, M	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
042600	2004	Stool	74, F	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
042679	2004	Blood	71, M	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042691	2004	Pus	36, M	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042721	2004	Sputum	83, F	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042728	2004	Sputum	48, M	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042763	2004	Stool	83, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
042781	2004	Sputum	61, M	+	+	*dfrA12-orfF-aadA2*; *aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042830	2004	Sputum	75, M	+	+	*aacA4-catB3-dfrA1* (noncoding)	−	−	F	GPTTcTo
042869	2004	Stool	66, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
042872	2004	Sputum	53, M	+	+	*sul3*	−	−	G	AAzCaCeCiGLPTTcToTs
042879	2004	Blood	71, F	+	+	*dfrA12-orfF-aadA2*; *dfrA17-aadA5*	−	−	B	AzCaCeCiGLPTTcToTs
042890	2004	Sputum	48, M	+	+	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042904	2004	Pus	28, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
042921	2004	Sputum	76, M	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042963	2004	Blood	86, F	+	+	*dfrA12-orfF-aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
042975	2004	Stool	63, M	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
043001	2004	Sputum	71, F	+	−	*aadA2*	−	−	B	AzCaCeCiGLPTTcToTs
051327	2005	Sputum	49, M	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
053203	2005	Blood	62, F	+	+	bla_{VIM-4}–*pse1*	−	−	H	AAzCaCeCiGLPTTcToTs
053375	2005	Sputum	33, F	+	+	*sul3*	−	−	G	AAzCaCeCiGLPTTcToTs
053505	2005	Stool	46, M	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054239	2005	Sputum	56, F	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054297	2005	Blood	38, F	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054313	2005	Sputum	55, M	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054348	2005	Blood	44, F	+	+	*sul3*	+	*dfrA1-sat1-aadA1*	G	AAzCaCeCiGLPTTcToTs
054372	2005	Sputum	43, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
054393	2005	Blood	57, F	−	−	−	+	*dfrA1-sat1-aadA1*	D	AAzCaCeCiGLPTTcToTs
054429	2005	Sputum	49, M	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054466	2005	Blood	60, F	+	+	bla_{VIM-4}–*pse1*	+	*dfrA1-sat1-aadA1*	H	AAzCaCeCiGLPTTcToTs
054498	2005	Sputum	51, M	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054574	2005	Blood	29, F	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs
054578	2005	Sputum	69, F	+	+	*sul3*	+	*dfrA1-sat1-aadA1*	G	AAzCaCeCiGLPTTcToTs
055180	2005	Sputum	66, F	−	−	−	+	*dfrA1-sat1-aadA1*	E	AAzCiGLPTTcToTs

[a] M, male; F, female.

[b] +, present; −, absent.

[c] A, amikacin; Az, aztreonam; Ca, ceftazidime; Ce, ceftriaxone; Ci, ciprofloxacin; G, gentamicin; L, levofloxacin; P, piperacillin; T, tetracycline; Tc, ticarcillin-clavulanic acid; To, tobramycin; Ts, trimethoprim-sulfamethoxazole.

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