

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or relevant characteristics ^a	Source or reference
Strains		
<i>E. coli</i>		
DH5α	F ⁻ ,φ80dlacZΔM15 Δ(<i>lacZYA-argF</i>)U169 <i>deoR</i> <i>recA1 endA1 hsdR17(rK⁻, mK⁺) phoA supE44 λ⁻ thi-1</i>	Invitrogen
CC118 λ-pir	Δ(ara-leu) <i>araD</i> Δ <i>lacX74 galE galK phoA20 thi-1 rpsE rpoB argE(Am) recA1 λ pir</i>	(3)
S17.1 λ-pir	<i>hsdR recA pro RP4-2 (Tc::Mu; Km::Tn7)(λ pir)</i>	(9)
<i>A. baumannii</i>		
A424	Clinical isolate	QMC ^a
A473	Clinical isolate	(7)
ATCC 19606	Clinical isolate	SGSC ^a
Plasmids		
pGEM®-T Easy	Cloning vector; Ap ^r	Promega
pUC18R6KminiTn7T-Gm	Ap ^r ; source of Gm ^r cassette	(1)
pISAbal1	1.7 kb fragment containing entire ISAbal1 inserted into pGEM®-T Easy	This study
pISAbal1Gm	<i>aacC1</i> cassette inserted downstream of ISAbal1 into pISAbal1	This study
pDS132	Suicide vector, R6K ori, <i>mobRP4, cat, sacB</i>	(5)
pDISAbal1Gm	<i>aacC1</i> -tagged ISAbal1 inserted into pDS132	This study

^a Abbreviations: QMC, Queen's Medical Centre, Nottingham; SGSC, *Salmonella* Genetic Stock Centre; Ap^r, ampicillin resistance; Gm^r, gentamicin resistance.

Table S2. ISAb11 and TnAbaR PCR survey results for the 148 strains representative of ≥19 Acinetobacter species investigated experimentally in this study

Strain	Species	<i>tnp</i> _{ISAb11} ^a	ISAb11 ^a	<i>tniA</i> ^a	<i>rpoB</i> ^a
A1	<i>A. baumannii</i>	-	-	+	+
A13	<i>A. baumannii</i>	-	-	-	+
A14	<i>A. baumannii</i>	-	-	+	+
A20	<i>A. baumannii</i>	-	-	-	+
A25	<i>A. baumannii</i>	-	-	+	+
A37	<i>A. baumannii</i>	-	-	-	+
A47	<i>A. baumannii</i>	-	-	+	ND
A52	<i>A. baumannii</i>	-	-	-	ND
A63	<i>A. baumannii</i>	-	-	+	+
A92	<i>A. baumannii</i>	-	-	-	+
A94	<i>A. baumannii</i>	-	-	-	+
A97	<i>A. baumannii</i>	-	-	-	+
A167	<i>A. baumannii</i>	-	-	-	+
A186	<i>A. baumannii</i>	-	-	+	ND
A187	<i>A. baumannii</i>		-	-	ND
A230	<i>A. baumannii</i>	-	-	+	ND
A329	<i>A. baumannii</i>	-	-	-	ND
A335	<i>A. baumannii</i>	-	-	+	ND
A343	<i>A. baumannii</i>	-	-	+	ND
A365	<i>A. baumannii</i>	-	-	+	ND
A367	<i>A. baumannii</i>	-	-	-	ND
A297	<i>A. baumannii</i>	-	-	+	+
A332	<i>A. baumannii</i>	-	-	+	+
A369	<i>A. baumannii</i>	-	-	+	+
A371	<i>A. baumannii</i>	-	-	+	+
A377	<i>A. baumannii</i>	-	-	-	+
A379	<i>A. baumannii</i>	-	-	-	ND
A380	<i>A. baumannii</i>	-	-	+	ND
A384	<i>A. baumannii</i>	-	-	+	+
A387	<i>A. baumannii</i>	-	-	+	+
A388	<i>A. baumannii</i>	-	-	+	+
A390	<i>A. baumannii</i>	-	-	+	ND
A392	<i>A. baumannii</i>	-	-	+	+
A397	<i>A. baumannii</i>	-	-	-	ND
A401	<i>A. baumannii</i>	-	-	-	+
A418	<i>A. baumannii</i>	-	-	+	+
A424	<i>A. baumannii</i>	-	-	+	+
A442	<i>A. baumannii</i>	-	-	+	+
A443	<i>A. baumannii</i>	-	-	+	+
A457	<i>A. baumannii</i>	-	-	-	+
A472	<i>A. baumannii</i>	-	-	+	+
A473	<i>A. baumannii</i>	+	+	+	+
A474	<i>A. baumannii</i>	-	-	+	ND
A479	<i>A. baumannii</i>	+	-	-	ND
A480	<i>A. baumannii</i>	-	-	+	ND

A481	<i>A. baumannii</i>	-	-	-	ND
A482	<i>A. baumannii</i>	-	-	+	ND
AB13	<i>A. baumannii</i>	-	-	+	ND
AB14	<i>A. baumannii</i>	-	-	+	ND
AS15	<i>A. baumannii</i>	-	-	+	ND
AB16	<i>A. baumannii</i>	-	-	+	ND
AB17	<i>A. baumannii</i>	-	-	+	ND
AB18	<i>A. baumannii</i>	-	-	-	ND
AB20	<i>A. baumannii</i>	-	-	+	+
AS20	<i>A. baumannii</i>	-	-	+	+
AB21	<i>A. baumannii</i>	-	-	+	ND
AB22	<i>A. baumannii</i>	-	-	+	ND
AL7	<i>A. baumannii</i>	+	-	-	+
AS27	<i>A. baumannii</i>	-	-	-	+
AS42	<i>A. baumannii</i>	+	+	-	+
KR1774	<i>A. baumannii</i>	-	-	-	+
AS44	<i>A. baumannii</i>	-	-	-	+
KR175	<i>A. baumannii</i>	-	-	+	+
AS45	<i>A. baumannii</i>	-	-	-	+
HPA10	<i>A. baylii/A. genomospecies 11</i>	-	-	-	+
HPA16	<i>A. baylii/A. genomospecies 11</i>	-	-	-	+
HPA22	<i>A. berezinae</i>	-	-	-	+
HPA26	<i>A. berezinae</i>	-	-	-	+
HPA3	<i>A. beijerinckii</i>	+	+	-	+
HPA21	<i>A. calcoaceticus</i>	-	-	-	+
HPA18	<i>A. genomospecies 13</i>	-	-	-	+
HPA23	<i>A. genomospecies 13</i>	+	+	-	+
HPA1	<i>A. genomospecies 16</i>	-	-	-	+
AS46	<i>A. genomospecies 15TU</i>	+	+	+	+
AS47	<i>A. genomospecies 15TU</i>	+	+	-	+
HPA8	<i>A. genomospecies 15TU</i>	+	+	-	+
HPA11	<i>A. gyllenbergsii</i>	+	+	-	+
HPA29	<i>A. haemolyticus</i>	-	-	-	+
HPA30	<i>A. haemolyticus</i>	+	-	-	+
HPA34	<i>A. haemolyticus</i>	+	-	-	+
AL1	<i>A. johnsonnii</i>	+	+	-	+
AS24	<i>A. johnsonnii</i>	+	+	-	+
HPA6	<i>A. johnsonnii</i>	-	-	-	+
HPA14	<i>A. johnsonnii</i>	+	+	-	+
HPA17	<i>A. johnsonnii</i>	+	+	-	+
HPA28	<i>A. johnsonnii</i>	+	+	-	+
AJ11	<i>A. junii</i>	+	+	-	+
AJ30	<i>A. junii</i>	+	+	-	+
AJ31	<i>A. junii</i>	+	+	+	+
AJ33	<i>A. junii</i>	+	+	-	+
AJ34	<i>A. junii</i>	+	+	-	+
AJ35	<i>A. junii</i>	+	+	+	+
HPA7	<i>A. junii</i>	+	+	-	+
HPA19	<i>A. junii</i>	+	+	-	+
AB3	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+

AL2	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AL5	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AL6	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AL27	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	ND
AL23	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS10	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS25	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS27	<i>A. Iwoffii/A. genomospecies 9</i>	-	-	-	+
AS28	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	+	+
AS38	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	+	+
AS43	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS48	<i>A. Iwoffii/A. genomospecies 9</i>	+	-	+	ND
AS49	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS50	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS51	<i>A. Iwoffii/A. genomospecies 9</i>	-	-	-	ND
AS52	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	+	+
AS53	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS54	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS55	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	+	+
AS56	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS57	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS58	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
AS59	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
KR1773	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
KR1776	<i>A. Iwoffii/A. genomospecies 9</i>	-	-	-	+
AS61	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
HPA12	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	+
HPA13	<i>A. Iwoffii/A. genomospecies 9</i>	+	-	+	+
HPA4	<i>A. nosocomialis</i> sp. nov.	-	-	-	+
HPA27	<i>A. nosocomialis</i> sp. nov.	-	-	-	+
AL40	<i>A. parvus</i>	+	+	-	+
AS62	<i>A. parvus</i>	+	+	-	+
AS63	<i>A. parvus</i>	-	-	-	+
A164	<i>A. pittii</i> sp. nov.	-	-	-	+
A215	<i>A. pittii</i> sp. nov.	-	-	-	+
A376	<i>A. pittii</i> sp. nov.	-	-	-	+
AB8	<i>A. pittii</i> sp. nov.	-	-	+	+
AB12	<i>A. pittii</i> sp. nov.	-	-	-	+
HPA5	<i>A. pittii</i> sp. nov.	-	-	-	+
HPA32	<i>A. pittii</i> sp. nov.	-	-	-	+
AS64	<i>A. radioresistens</i>	-	-	+	+
HPA2	<i>A. radioresistens</i>	-	-	-	+
HPA33	<i>A. radioresistens</i>	+	+	-	+
AS65	<i>A. schindleri</i>	+	-	+	+
HPA20	<i>A. schindleri</i>	-	-	-	+
HPA31	<i>A. schindleri</i>	-	-	-	+
AS26	<i>A. ursingii</i>	+	+	-	+
HPA25A	<i>A. ursingii</i>	-	-	-	+
HPA15	<i>A. ursingii</i>	-	-	-	+
HPA9	<i>A. ursingii</i>	-	-	-	+

AS4	<i>A. ursingii</i> group	-	-	-	+
AS39	<i>A. ursingii</i> group	-	-	+	+
KR1778	<i>A. ursingii</i> group	-	-	-	+

^a The following PCR primers were used: *tnp*_{ISAb*a*11}, *tnp*-F/*tnp*-R; ISAb*a*11, ISAb*a*11-F/ISAb*a*11-R; *tniA*, *tniAF*/*tniA*; *rpoB*, Ac696F/Ac1093R. The *tnp*_{ISAb*a*11}-primers amplify a 300 bp internal segment of ISAb*a*11, while the ISAb*a*11-primers amplify the entire 1101 bp element. *rpoB* PCR amplicons were sequenced to inform about speciation. ND, not done.

Table S3. ISAb11 and TnAbaR PCR survey results for the 48 genome sequences representative of ≥9 *Acinetobacter* species investigated by BlastN and *in silico* PCR analysis in this study

Strain	Species	<i>tnp</i> _{ISAb11} ^a	ISAb11 ^a	<i>tniA</i> ^a	Genome status	GenBank accession no.
1656-2	<i>A. baumannii</i>	-	-	+	assembled	CP001921
3909	<i>A. baumannii</i>	-	-	-	not assembled	AEOZ00000000
3990	<i>A. baumannii</i>	-	-	-	not assembled	AOY00000000
4190	<i>A. baumannii</i>	-	-	-	not assembled	AEPA00000000
6013113	<i>A. baumannii</i>	+	-	-	not assembled	ACYR02000038
6014059	<i>A. baumannii</i>	-	-	+	not assembled	ACYS02000127
A118	<i>A. baumannii</i>	-	-	-	not assembled	AEOW00000000
AB0057	<i>A. baumannii</i>	-	-	+	assembled	CP001182
AB056	<i>A. baumannii</i>	-	-	+	not assembled	ADGZ01000825
AB058	<i>A. baumannii</i>	-	-	+	not assembled	ADHA01000065
AB059	<i>A. baumannii</i>	-	-	+	not assembled	ADHB01000787
AB210	<i>A. baumannii</i>	-	-	+	not assembled	AOX01000067
AB307-0294	<i>A. baumannii</i>	-	-	-	assembled	CP001172
AB900	<i>A. baumannii</i>	-	-	-	not assembled	ABXK00000000
ABNIH1	<i>A. baumannii</i>	-	-	-	not assembled	AFSZ00000000
ABNIH2	<i>A. baumannii</i>	-	-	+	not assembled	AFTA01000065
ABNIH3	<i>A. baumannii</i>	-	-	+	not assembled	AFTB01000233
ABNIH4	<i>A. baumannii</i>	-	-	-	not assembled	AFTC00000000
ACICU	<i>A. baumannii</i>	-	-	-	assembled	CP000863
ATCC 17978	<i>A. baumannii</i>	+	+	+	not assembled	CP000521
ATCC 19606	<i>A. baumannii</i>	+	+	-	not assembled	ACQB00000000
AYE	<i>A. baumannii</i>	-	-	+	assembled	CU459141
D1279779	<i>A. baumannii</i>	-	-	-	not assembled	AERZ00000000
DR1	<i>A. baumannii</i>	-	-	-	assembled	CP002080
MDR-TJ	<i>A. baumannii</i>	-	-	+	assembled	AOOE01000001
MDR-ZJ06	<i>A. baumannii</i>	-	-	+	assembled	CP001937
Naval-18	<i>A. baumannii</i>	-	-	+	assembled	AFDA01000030
Naval-81	<i>A. baumannii</i>	-	-	-	not assembled	AFDB00000000
OIFC032	<i>A. baumannii</i>	-	-	+	not assembled	AFCZ01000012
SDF	<i>A. baumannii</i>	-	-	-	assembled	CU468230
TCDC-AB0715	<i>A. baumannii</i>	-	-	+	assembled	CP002522
UMB001	<i>A. baumannii</i>	-	-	-	not assembled	AEBK00000000
UMB002	<i>A. baumannii</i>	-	-	-	not assembled	AEBL00000000
UMB003	<i>A. baumannii</i>	-	-	-	not assembled	AEBM00000000
WM99c	<i>A. baumannii</i>	-	-	+	not assembled	AERY01000108
PHEA-2	<i>A. calcoaceticus</i>	-	-	-	assembled	CP002177
RUH2202	<i>A. calcoaceticus</i>	-	-	-	not assembled	ABCK00000000
ATCC 19194	<i>A. haemolyticus</i>	+	-	-	not assembled	ADMTO1000000
ATCC 27244	<i>A. haemolyticus</i>	+	-	-	not assembled	ABYN01000000
ADP1	<i>A. johnsonii</i>	-	-	-	assembled	CR543861
SH046	<i>A. johnsonii</i>	+	-	-	not assembled	ACPL01000000
SH205	<i>A. junii</i>	-	-	-	not assembled	ACPM00000000
SH145	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	not assembled	ACPN01000000
WJ10621	<i>A. Iwoffii/A. genomospecies 9</i>	+	+	-	not assembled	AFQY01000001
SH024	<i>A. pittii</i> sp. nov.	-	-	-	not assembled	ADCH00000000
RUH2624	<i>A. nosocomialis</i> sp. nov.	-	-	-	not assembled	ACQF00000000
SH164	<i>A. radioresistens</i>	-	-	-	not assembled	ACPO00000000
SK82	<i>A. radioresistens</i>	-	-	-	not assembled	ACVR00000000

^a The following PCR primers were used: *tnp*_{ISAb11}, *tnp*-F/*tnp*-R; ISAb11, ISAb11-F/ ISAb11-R; *tniA*, *tniAF*/*tniAR*.

Table S4. Primers used in this study

Name	Sequence 5'- 3'	Source	Target (Purpose)
tniA-R2	CGAAGACGACAGCAGGTACA	This study	<i>tniA</i> junction
tnp-F	CAAAGATCCCCCAAAGCTGG	This study	
tnp-R	ATCAGGGTCAAGTGGTCTGG	This study	
orf1-F	GTTGAATCGACCCTTGAGC	This study	
orf1-R	CCCTCATAACCGACAACCAC	This study	TnAbaR <i>orfI</i>
Gm-F-BamHI	CGGGATCC GAATTAGCTCAAAAGCGCTCTGA ^a	This study	
Gm-R-BamHI	CGGGATCC GAATTGGGGATCTTGAAGTTCC ^a	This study	<i>aacC1</i> cassette
ISAb11-F	TAGGACTTACGCATTGACG	This study	
ISAb11-R	TAGGACTTACGCACTATCATTAT	This study	ISAb11
ISAb11-InF	CGGGATCC TCATTATAGATTCTCTGTGGTAGC ^a	This study	pDSISAb11
ISAb11-InR	CGGGATCC CAGTGCATAAGCCTACATATAATC ^a	This study	(inverse-PCR)
DFISAb11-1	GTAAGCCATCAAGGCTGAAA	This study	
DFISAb11-2	GGCAAGTTGTGTCAGCTAA	This study	
pDS-F	GGAACACTTAACGGCTGAC	This study	
pDS-R	GGATCGATCCTCTCAGAGTC	This study	pDS junctions
sacB-F	CGGCATTTCTTTGCGTTT	Kochar <i>et al.</i>	
sacB-R	AGGAACTTCAAGATCCCCAATTGCTTTAGGCC CGTAGTCTGC	Kochar <i>et al.</i>	<i>sacB</i>
4R	AATCGATCGGGTCGAGTAAC	(8)	<i>comM</i> junction
aacC1F	GACATAAGCCTGTTGGTT	(4)	
aacC1R	CTCCGAACTCACGACCGA	(4)	<i>aacC1</i>
oxa51a	CTAATAATTGATCTACTCAAG	(6)	
oxa51b	CCAGTGGATGGATGGATAGATTATC	(6)	<i>bla</i> _{OXA-51-like}
Ac696F	TAYCGYAAAGAYTTGAAAGAAG	(2)	
Ac1093R	CMACACCYTTGTTMCCRTGA	(2)	<i>rpoB</i>
tniAF	CATCCCCAATCGTTAAATGG	(7)	
tniAR	TTTCTTTRCGCTTCGAT	(7)	<i>tniA</i>

^a Residues shown in bold correspond to primer-incorporated *BamHI* sites.

Table S5. Results of genomic walking analysis on eleven ISAb_a11-positive *Acinetobacter* spp. strains ^a

GenBank accession no. ^b	Species	Strain	TIR ^c	Length of non-ISAb _a 11 sequence	Target gene (site) /protein	Species (strain)	GenBank ID	Identity BlastX	Identity BlastN
JN819186	<i>A. baumannii</i>	ATCC 19606	IRL	699 bp	Upstream of HMPREF0010_03298/hypothetical protein	<i>A. baumannii</i> ATCC 19606	EEX02178	99%	99%
JN819187	<i>A. baumannii</i>	ATCC 19606	IRR	232 bp	Upstream of HMPREF0010_03297/phospholipase C	<i>A. baumannii</i> ATCC 19606	ZP_05829914	97%	98%
JN819188	<i>A. baumannii</i>	A479	IRL	792 bp	ISAb _a 1	<i>A. baumannii</i> AYE	YP_001712482	100%	100%
JN819189	<i>A. baumannii</i>	A479	IRR	549 bp	AB57_3457/enoyl-CoA hydratase	<i>A. baumannii</i> AYE	YP_002320761	98%	99%
JN819190	<i>A. junii</i>	AJ11	IRL	802 bp	Upstream of HMPREF0026_01257/hypothetical protein	<i>A. junii</i> SH206	EEY93981	100%	100%
JN819191	<i>A. lwoffii/A. gs. 9</i>	AL1	IRL	256 bp	Upstream of HMPREF0017_01038/quinone oxidoreductase	<i>A. lwoffii/A. gs. 9</i> SH145	EEY90112	100%	100%
JN819193	<i>A. lwoffii/A. gs. 9</i>	AL2	IRR	368 bp	Upstream of HMPREF0017_02635/sulfite reductase	<i>A. lwoffii/A. gs. 9</i> SH145	EEY88689	100%	100%
JN819192	<i>A. lwoffii/A. gs. 9</i>	AL2	IRL	557 bp	HMPREF0017_02339/membrane protein	<i>A. lwoffii/A. gs. 9</i> SH145	EEY89069	97%	90%
JN819194	<i>A. lwoffii/A. gs. 9</i>	AL5	IRR	308 bp	HMPREF0026_02009/transposase	<i>A. junii</i> SH205	ZP_06066902	98%	97%
JN819195	<i>A. lwoffii/A. gs. 9</i>	AL5	IRL	536 bp	HMPREF0017_01092/esterase	<i>A. lwoffii/A. gs. 9</i> SH145	EEY90166	96%	97%
JN819198	<i>A. lwoffii/A. gs. 9</i>	AS25	IRL	135 bp	NA	<i>A. lwoffii/A. gs. 9</i> WJ10621	AFQY01000001	NA	95%
JN819196	<i>A. lwoffii/A. gs. 9</i>	AS10	IRR	472 bp	Downstream of HMPREF0017_00131/phosphate acetyltransferase	<i>A. lwoffii/A. gs. 9</i> SH145	EEY91118	99%	90%
JN819197	<i>A. lwoffii/A. gs. 9</i>	AS10	IRL	368 bp	HMPREF0017_01411/RNA helicase	<i>A. lwoffii/A. gs. 9</i> SH145	EEY89994	98%	92%

JN819199	<i>A. baumannii</i>	A424-BR3	IRR, IRL	678 bp	pMMA2_02/TonB-dependent receptor	<i>A. baumannii</i> AYE	ACV72167	98%	100%
JN819200	<i>A. baumannii</i>	A424-BR2	IRR, IRL	1717 bp	Downstream of ABAYE2100/ ABC efflux protein	<i>A. baumannii</i> AYE	CAM86974	100%	100%
JN819201	<i>A. baumannii</i>	A424-BR1	IRR, IRL	1502 bp	Upstream of FG00266.1/ hypothetical protein	<i>Gibbersella zaeae</i> PH-1	XP_380442	26%	NS

^a NA, not applicable; NS, not significant; gs., genomospecies. ^b Genomic walking sequence data generated in this study has been deposited with GenBank and is available via the accession numbers listed. ^c Flanking sequence data was generated for the TIR end shown.

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