Table 3: Genes and their location in the genome predicted to be involved in Resistance and stress tolerance

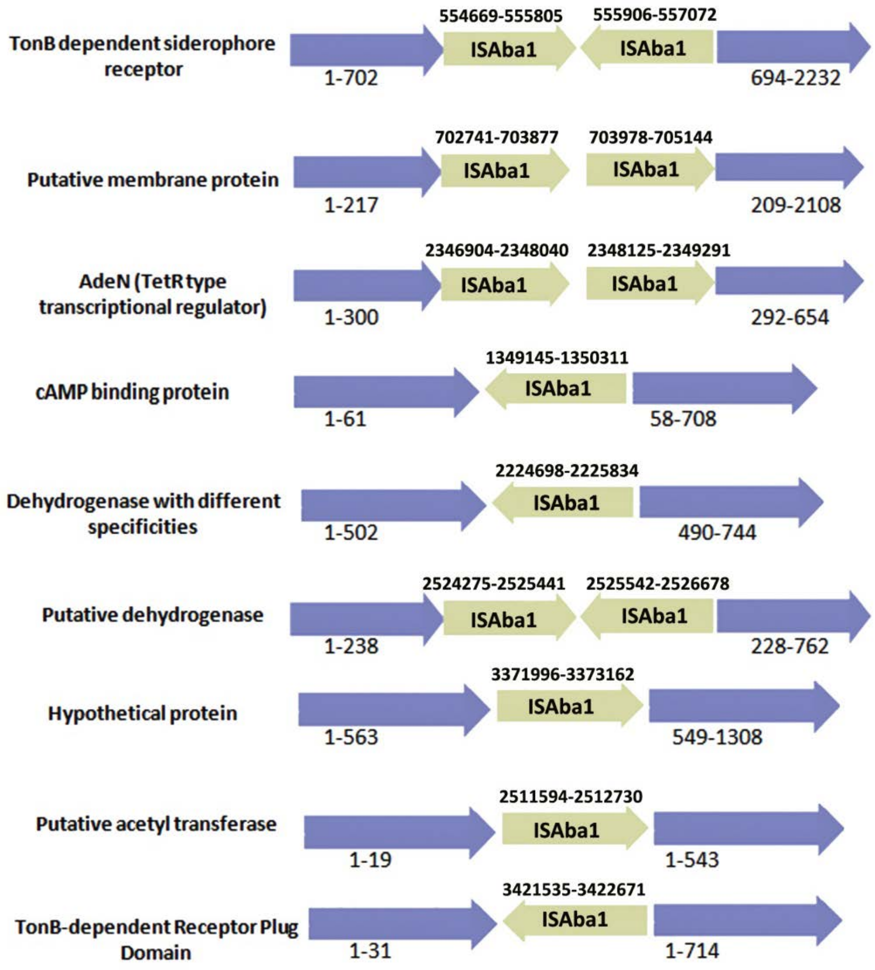
Sl. No	Drug class	Gene product	Gene	Function	Locus/ Protein ID
ResFinder analysis					
	Aminoglycosides	Streptomycin	*aadA*	Streptomycin 3'- adenylyltransferase	AHJ91630.1
	β-Lactam	class D β–lactamase	*bla*$_{OXA-66}$	beta-lactamase	AHJ93068.1
		class C β–lactamase	*ampC*	beta-lactamase	AHJ94023.1
		Gentamicin	*Grm*	16S rRNA methyl transferase	AHJ93859.1
	Macrolide		*mph(E)*	macrolide 2'-phosphotransferase	AHJ93855.1
			msr(E)	ABC transporter	AHJ93856.1
		Erythromycin	*msrA*	ATP-binding protein	AHJ93856.1
Manual search for resistance related genes					
	Efflux pumps	ABC (ATP binding cassette type)	*mlaD*	Phospholipid ABC transporter-binding protein	AHJ94898.1, AHJ94724.1
			mlaE	Phospholipid ABC transporter permease	AHJ94725.1, AHJ94899.1
			bepE	Membrane transporter	AHJ93962.1
			yrbF	Transporter - resistance to organic solvents	AHJ94900.1, AHJ94726.1
		RND (Resistance-Nodulation-Division)		RND Type Efflux Pump Involved In Aminoglycoside Resistance	AHJ94889.1, AHJ91476.1, AHJ93342.1, AHJ93952.1, AHJ94715.1, AHJ91475.1, AHJ92220.1, AHJ93363.1, AHJ93961.1, AHJ94418.1, AHJ95243.1, AHJ94345.1
			arpC	outer membrane protein - Antibiotic efflux	AHJ93963.1
		MFS (Major Facilitator Superfamily)		mutlidrug resistance protein	AHJ93846.1
			emrB	Multidrug resistance protein B	AHJ93362.1
			Norm	Probable multidrug resistance protein norm	AHJ91706.1, AHJ95212.1
			mdtD	Putative multidrug resistance protein mdtD	AHJ91682.1
			ybdA	Facilitator Superfamily Protein	AHJ92081.1
			emrY	Facilitator Superfamily Protein	AHJ92221.1, AHJ93399.1
			nepI	Purine ribonucleoside efflux	AHJ91658.1
		SMR (Small Multidrug Resistance)	*mdfA*	Efflux – Broad spectrum of drugs	AHJ94770.1, AHJ94944.1
			emrB	Multidrug resistance protein B	AHJ92308.1
			ydhJ	Multidrug resistance efflux pump	AHJ91809.1

	MATE (multidrug and toxic compound extrusion)	*norM*	Probable multidrug resistance protein norM	AHJ91879.1
		yoeA	Probable multidrug resistance protein yoeA	AHJ95166.1
	Chloramphenicol	*catB2*	Acetyl transferase	AHJ94372.1
Glycopeptide	Bleomycin		Glyoxalase /Dioxygenase	AHJ92301.1, AHJ95208.1
Polypeptide	Bacitracin	*ybjG*	Undecaprenyl diphosphatase	AHJ91500.1
		uppP	Undecaprenyl pyrophosphate phosphatase	AHJ94448.1
	Polymyxin B	*eptA*	Phosphoethanolamine transferase eptA	AHJ92628.1
	Albicidin	*zntR*	Albicidin resistance protein	AHJ92771.1
Sulpha drugs		*bcr*	Sulfonamide And Bicyclomycin Resistance	AHJ94372.1
		folP-A	Folic acid precursor synthesis	AHJ92485.1
Metals	Arsenic	*arsR*	Arsenical resistance operon repressor	AHJ93001.1
		arsC	Reduction of arsenate (As(V)) to arsenite(As(III))	AHJ93000.1
		arsB	Arsenite resistance protein ArsB	AHJ93002.1
	Copper	*pcoB*	Copper resistance protein B	AHJ92138.1
		cutF	Copper Resistance Lipoprotein NlpE	AHJ92534.1
		copA	Copper resistance protein A homolog	AHJ92137.1
	Co/Zn/Cd Efflux		Cation Efflux Protein	AHJ92139.1
		CzcC	Cobalt-Zinc-Cadmium Resistance Protein	AHJ95012.1, AHJ95012.1
		CzcN	Cobalt-zinc-cadmium resistance protein	AHJ95015.1
		czcA	Cobalt-zinc-cadmium resistance protein	AHJ95010.1, AHJ94835.1
			Cation Efflux Protein; Co/Zn/Cd Efflux System Component	AHJ92640.1
	Tellurium	*yoaE*	tellurium resistance	AHJ91684.1
			Tellurite resistance protein – permease	AHJ91524.1
		alx	tellurium resistance	AHJ91499.1
	Mercury	*merA*	MerA protein is responsible for volatilizing mercury as Hg(0)	AHJ94611.1
	Chromate	*chrA1*	Chromate transport protein	AHJ93260.1
Inorganic compounds and dyes	Toluene	*ttg2D*	Toluene tolerance protein	AHJ94723.1, AHJ94897.1
	Quaternary ammonium compound	*qacF*	Quaternary ammonium compound-resistance protein qacF	AHJ93954.1
		sugE	Quaternary ammonium compound-resistance protein	AHJ92140.1
		emrE	Quaternary ammonium compound-resistance protein	AHJ94527.1
	Acriflavine	*acrB*	Acriflavine resistance protein B	AHJ93338.1
		acrA	Acriflavine resistance protein A	AHJ94417.1
	DNA-Damaging Agents	*uspA*	Required For Resistance To DNA-Damaging Agents	AHJ94373.1
	Azleucine	*ygaZ*	Predicted branched-chain amino acid permease	AHJ95075.1, AHJ93023.1, AHJ93978.1

	Acid	*ydeP*	Probably involved in acid resistance	AHJ94327.1
	Camphor	*crcB*	Protein CrcB homolog	AHJ91873.1
Stress tolerance	Peroxide	*ohrR*	Organic hydroperoxide resistance transcriptional regulator	AHJ93439.1
		perR	Peroxide resistance protein per	AHJ92488.1
	Serum resistance	*yadA*	Responsible for agglutination, serum resistance, complement inactivation and phagocytosis resistance	
	Osmotic stress	*BASYS01690*	OsmC-Like Protein; Osmotically Inducible Protein	AHJ93074.1
		ktrA	ktrAB potassium uptake transporter	AHJ93546.1
		ktrB	ktrAB potassium uptake transporter	AHJ93547.1
Transcriptional regulators of Multidrug resistance		*ada*	The methylation of Ada by methylphosphotriesters in DNA leads to its activation as a transcriptional regulator that activates the transcription of its own gene, ada, and other alkylation resistance genes	AHJ92307.1
		marR	Transcriptional Repressor MprA; Transcriptional Repressor For Multidrug Resistance Pump; The Activation Of Both Antibiotic Resistance And Oxidative Stress Genes	AHJ91804.1
		vqsM	Putative regulatory proteins which are implicated in quorum sensing, virulence and multidrug resistance	AHJ93494.1
		ybfI	Transcriptional Activator Of Genes Involved In The Multiple Antibiotic Resistance (Mar) Phenotype. It Can Also Activate Genes Such As Soda, Zwf And Micf	AHJ93134.1
		mrpG	Multiple resistance and pH homeostasis protein G	AHJ93884.1
		tetR	TetR is the repressor of the tetracycline resistance element	AHJ91827.1
		ohrR	Organic hydroperoxide resistance transcriptional regulator	AHJ91707.1
		mprA	Transcriptional Regulator; Transcriptional Repressor MprA; Transcriptional Repressor Of For Multidrug Resistance Pump	AHJ92107.1

Figure 5

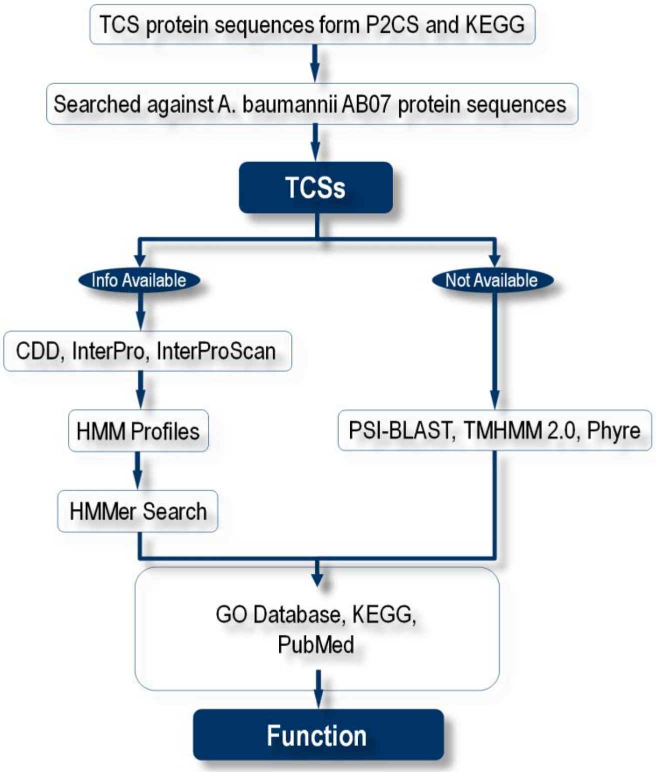
IS*Aba1* insertions in the genome of *A. baumannii* PKAB07. The genetic co-ordinates of genes in the PKAB07 are illustrated where IS*Aba1* insertions were present either in the upstream region or internal to the genes.



Methodology for detection of Two-component system (TCS) in *A. baumannii* PKAB07

TCS members were identified by an exhaustive search of the entire gene set of *A. baumannii* PKAB07 to identify TCS members against the Conserved Domain Database for HK (Histidine kinase), RR (Response regulator) and PT (Phospho-transferase) domains. The genes that were found were searched against the P2CS database[1]. The probable hits were searched against InterProScan and The InterPro database to confirm the TCS members; HMM profiles corresponding to identify domains were used for HMMER searches. BLAST, Pfam, TMHMM 2.0 and Phyre fold recognition tool were used to predict the function of uncharacterized proteins (see flow chart below). Interacting partners were identified or predicted by extensive literature mining involving *Acinetobacter* or its close relatives *Pseudomonas*, *Burkholderia* and *Serratia* using STRING protein-protein interaction database, Gene Ontology Annotation (GOA) Database and the PubMed.

FLOW CHART



Reference:

1.Barakat, M., Ortet, P., & Whitworth, D. E. (2011). P2CS: a database of prokaryotic two-component systems. *Nucleic acids research*, 39(suppl 1), D771-D776.

Table - 1

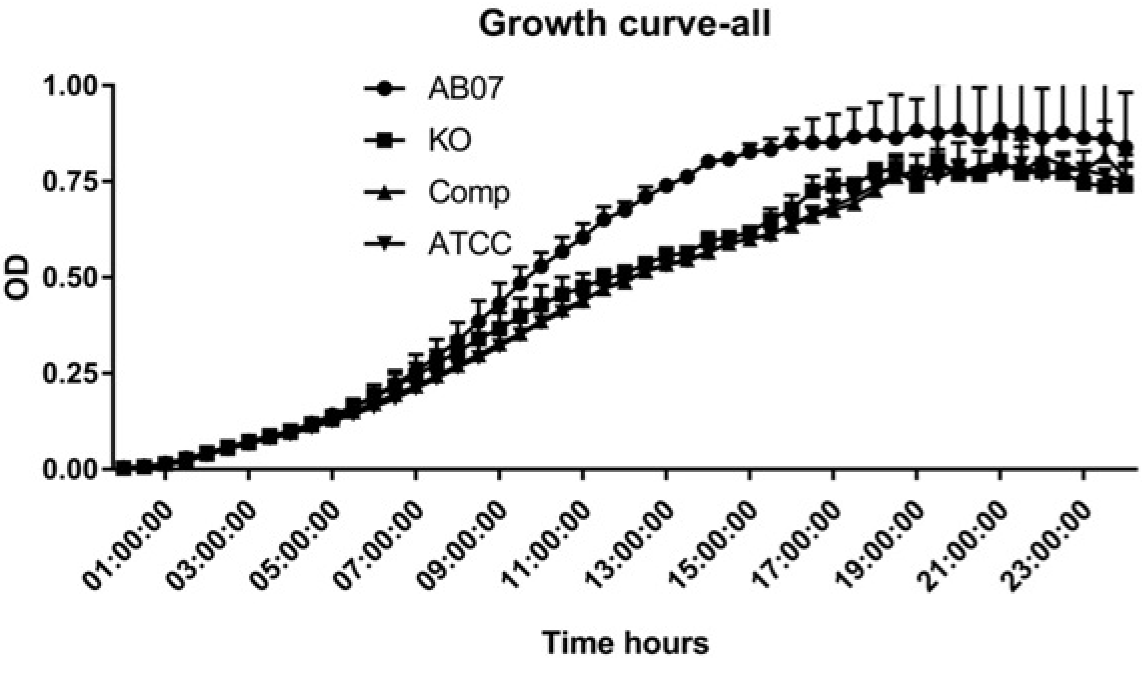
Main features of *A. baumannii* PKAB07 Genome

GenBank ID	CP006963.1
Genome size	4.23mb
Coding sequences	3772
Core genome	2.25mb (53%)
Accessory genome	1.98mb (47%)
Genes in core genome (Important broad categories)	Genes for heavy metal resistance, resistance to fluoroquinolones, β-lactamases and MDR efflux pumps
Genes in accessory genome (Important broad categories)	β-lactamases, aminoglycoside modification enzymes, tetracycline resistance, transposable elements, phages and pro-phages, genes for biofilm formation and chaperone-usher pili pathway
#IS*Aba1* copies	26

Cell Viability Assay- AO/EB staining

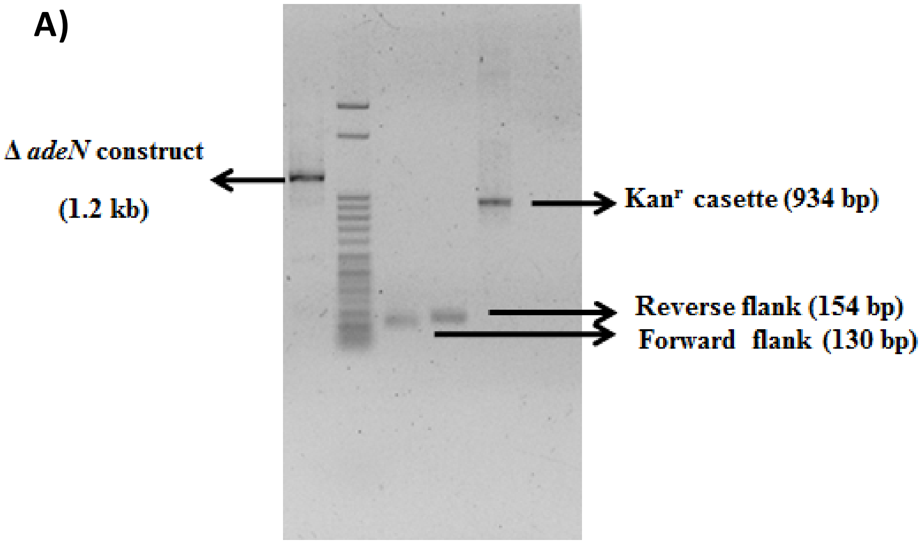
The A549 cells were seeded in a 12-well plate and the spent medium was replaced with fresh DMEM free of antibiotics. *A. baumannii* cells were grown overnight in LB broth, collected and re-suspended in DMEM without antibiotics. The bacterial cells were then added to the A549 monolayer at an MOI of **100**. The plates were then incubated for 24 h following which the medium was removed, washed twice with PBS and 20 µl of AO/EB stain was added into the wells.The cells were observed under the fluorescent microscope B-2A filter (Nikon Eclipse TS100, Japan).

S 1

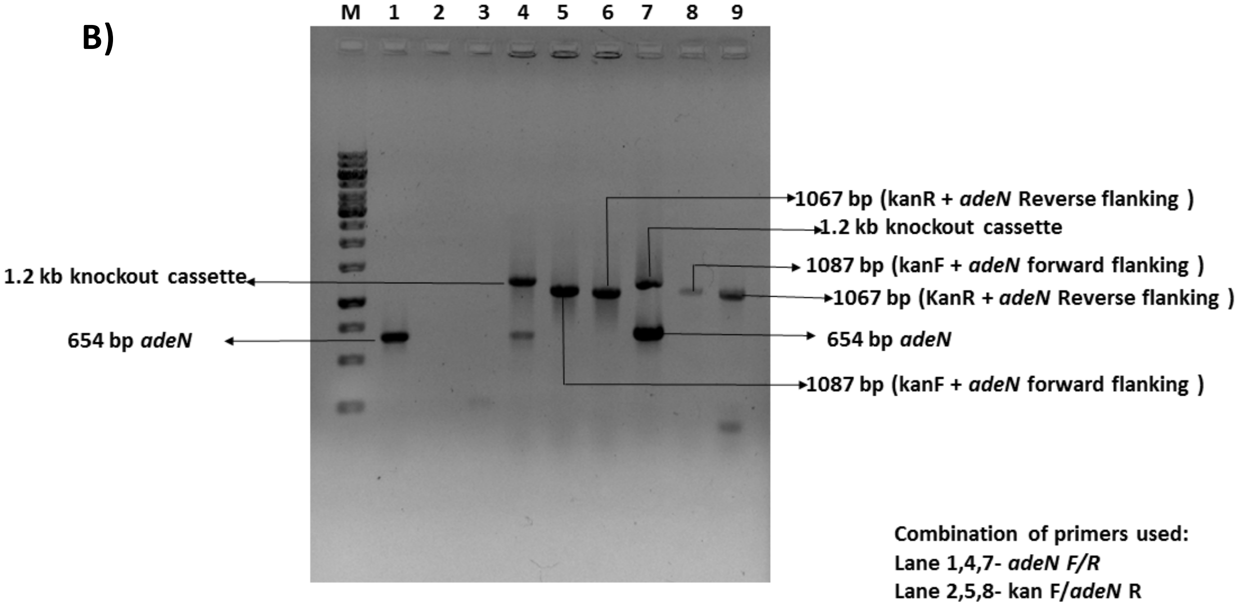


S1 – Growth rate analysis of *A. baumannii* strains

S 2



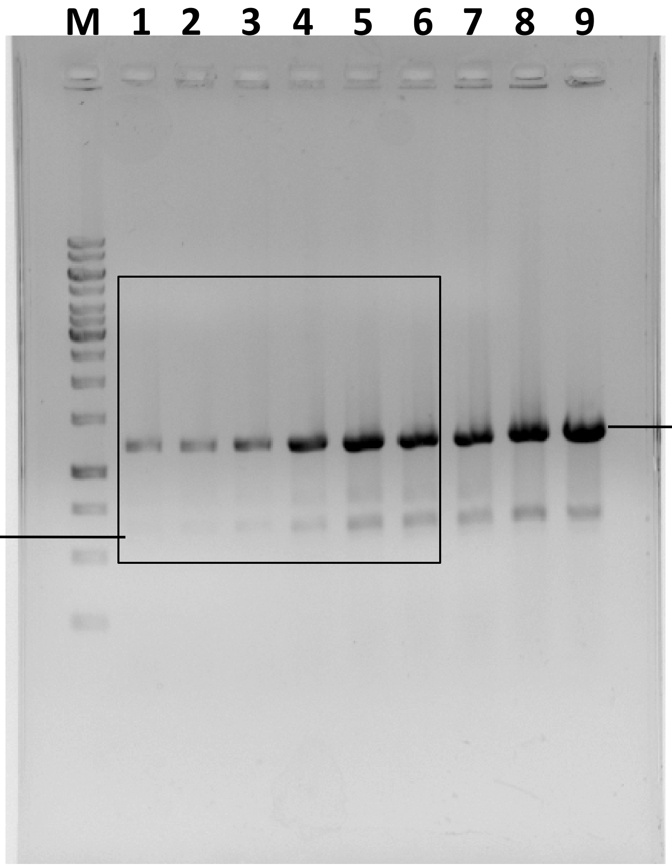
Amplicons of *adeN* flanking regions, Kanamycin cassette and Δ*adeN* construct (Kanamycin cassette flanked with *adeN*). Lane 1: Δ*adeN* construct carrying upstream and downstream regions of *adeN* and kanamycin resistance cassette; Lane M: 3Kb DNA ladder (Sigma Aldrich, USA), Lane 2, 3 & 4 Forward flanking, reverse flanking regions of *adeN* and kanamycin resistance cassette.



Combination of primers used:
Lane 1,4,7- *adeN F/R*
Lane 2,5,8- kan F/*adeN* R
Lane 3,6,9- *adeN* F/kan R

Lane M: Marker, Lane 1-3:ATCC, Lane 4-6: Knockout, Lane7-9 : Complement

S 3



Δ *adeN* construct

Window period – during this period all our experiments were conducted that is well ahead of critical point of spontaneous reversion of minuscule subpopulation of mutant started emerging out

Time lapse Colony PCR

M – Maker ; Lane 1 to 10 Cultures of Δ*adeN* strain grown at 2h, 4h, 6h, 8h, 10h, 12h, 24h and 48h subjected to Time lapse colony PCR to amplify the knock out construct of *adeN* (1.2kb)