

Fig S1 Potentiation of IBG partnered with amikacin, gentamicin against *S. aureus*.

a, The microdilution chequerboard assays of IBG and amikacin against *S. aureus* ATCC 29213. b, The microdilution chequerboard assays of IBG and amikacin against MRSA ATCC 43300. c, The microdilution chequerboard assays of IBG and gentamicin against *S. aureus* ATCC 29213. d, The microdilution chequerboard assays of IBG and gentamicin against MRSA ATCC 43300.

IBG, isopropoxy benzene guanidine; VAN, vancomycin; AMI, amikacin; GEN, gentamicin; The colour gradient represents the bacterial cell density estimated by OD₆₀₀ of biological replicates.

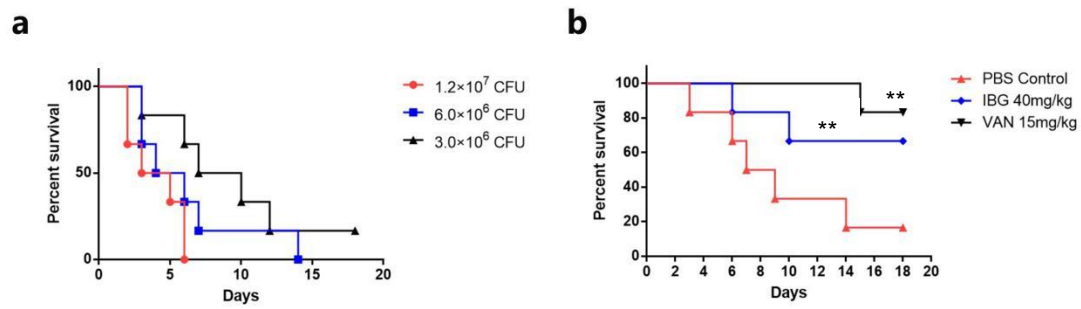


Fig S2 IBG is effective in a mouse model of MRSA septicemic infection

a, Different MRSA infection doses in mouse septicemic model b, Survival curves showing efficacy in a mouse septicemic infection model when treated with different drugs. A log rank test was performed using 95% confidence intervals and the statistical significance was calculated in order to compare treated to control groups. P values of ($* \leq 0.05$) ($**P \leq 0.01$) are considered as significant.

Supplementary Table S1: Activity of IBG against Gram-negative bacteria

Organism and genotype	IBG MIC ($\mu\text{g/ml}$)
<i>Escherichia coli</i> ATCC25922	>256
<i>Salmonella</i> ATCC14028	>256
<i>Klebsiella pneumoniae</i> ATCC 4752	>256
<i>Acinetobacter baumannii</i> ATCC 19606	>256
<i>Pasteurella multocida</i> CVCC399	>256
<i>Haemophilus parasuis</i> CVCC13R	>256
<i>Serratia marcescens</i> CVCC52721	>256
Clinical <i>Pseudomonas aeruginosa</i>	>256

Supplementary Table 2 Amino acid changes located in open reading frames after *in vitro* selection with IBG

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP1	58	127787	missense variant	<i>pnp_1</i>	polyribonucleotide nucleotidyltransferase	247C>G	Arg83Gly
SP1	58	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP1	58	31303	missense variant	<i>walR</i>	Transcriptional regulatory protein walR	319C>T	Arg107Cys
SP1	58	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase PcrA	761A>G	Asp254Gly
SP1	58	34705	missense variant	<i>ybiV</i>	sugar phosphatase YbiV	527C>T	Ser176Leu
SP1	58	43553	missense variant	<i>Org1_01695</i>	hypothetical protein	95C>A	Pro32Gln
SP1	58	61600	missense variant	<i>Org1_01489</i>	hypothetical protein	206G>C	Gly69Ala
SP1	58	76543	missense variant	<i>Org1_01939</i>	putative universal stress protein	247G>A	Val83Ile
SP1	58	85845	missense variant	<i>Org1_00143</i>	hypothetical protein	42G>A	Met14Ile
SP1	99	1594	stop gained	<i>rsmG</i>	ribosomal RNA small subunit methyltransferase G	153T>G	Tyr51*

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP1	99	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP1	99	28879	missense variant	<i>dtd3</i>	D-aminoacyl-tRNA deacylase	469G>A	Gly157Ser
SP1	99	31189	missense variant	<i>walR</i>	transcriptional regulatory protein walR	205C>T	Arg69Cys
SP1	99	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase PcrA	761A>G	Asp254Gly
SP1	99	43795	stop gained	<i>Org1_01695</i>	hypothetical protein	665G>A	Trp222*
SP1	99	61414	frameshift variant	<i>Org1_01489</i>	hypothetical protein	22_41delTTTCGAAA AGAAATTCTACA	Phe8fs
SP1	99	65156	missense variant	<i>prkC</i>	serine/threonine-protein kinase PrkC	1610A>T	Lys537Ile
SP1	99	82809	missense variant	<i>lip2</i>	lipase 2	1219G>A	Val407Ile
SP1	99	128200	missense variant	<i>mnhG1</i>	Na(+)/H(+) antiporter subunit G1	74C>A	Ala25Asp

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP2	58	8863	missense variant	<i>rpoE</i>	putative DNA-directed RNA polymerase subunit delta	189C>A	Asn63Lys
SP2	58	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP2	58	31303	missense variant	<i>walR</i>	transcriptional regulatory protein <i>walR</i>	319C>T	Arg107Cys
SP2	58	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase PcrA	761A>G	Asp254Gly
SP2	58	34705	missense variant	<i>ybiV</i>	Sugar phosphatase YbiV	527C>T	Ser176Leu
SP1	58	43553	missense variant	<i>Org1_01695</i>	hypothetical protein	95C>A	Pro32Gln
SP1	58	51219	missense variant	<i>Org1_01701</i>	hypothetical protein	29G>C	Gly10Ala
SP1	58	61600	missense variant	<i>Org1_01489</i>	hypothetical protein	206G>C	Gly69Ala
SP2	58	76543	missense variant	<i>Org1_01939</i>	putative universal stress protein	247G>A	Val83Ile
SP2	58	84845	missense varian	<i>Org1_00143</i>	hypothetical protein	42G>A	Met14Ile
SP2	58	127787	missense variant	<i>pnp_1</i>	polyribonucleotide nucleotidyltransferase	247C>G	Arg83Gly

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP2	100	20011	frameshift variant	<i>nusG</i>	transcription termination/ antitermination protein NusG	193delA	Thr65fs
SP2	100	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP2	100	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase PcrA	761A>G	Asp254Gly
SP2	100	33662	missense variant	<i>isdG_2</i>	heme oxygenase (staphylobilin-producing)	409T>C	Tyr137His
SP2	100	34665	frameshift variant	<i>Org1_00312</i>	hypothetical protein	1150dupT	Ser384fs
SP2	100	433559	missense variant	<i>Org1_01695</i>	hypothetical protein	901G>T	Val301Leu
SP3	34	1594	stop gained	<i>rsmG</i>	ribosomal RNA small subunit methyltransferase G	153T>G	Tyr51*
SP3	34	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP3	34	28879	missense variant	<i>dtd3</i>	D-aminoacyl-tRNA deacylase	469G>A	Gly157Ser

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP3	34	31189	missense variant	<i>walR</i>	Transcriptional regulatory protein walR	205C>T	Arg69Cys
SP3	34	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase PcrA	761A>G	Asp254Gly
SP3	34	61489	missense variant	<i>Org1_01489</i>	hypothetical protein	95C>A	Pro32Gln
SP3	34	82809	missense variant	<i>lip2</i>	lipase 2	1219G>A	Val407Ile
SP3	66	10208	missense variant	<i>Org1_00874</i>	hypothetical protein	82G>T	Ala28Ser
SP3	66	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP3	66	28378	missense variant	<i>Org1_00657</i>	hypothetical protein	500C>T	Thr167Ile
SP3	66	31303	missense variant	<i>walR</i>	transcriptional regulatory protein walR	319C>T	Arg107Cys
SP3	66	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase PcrA	761A>G	Asp254Gly
SP3	66	34705	missense variant	<i>ybiV</i>	sugar phosphatase YbiV	527C>T	Ser176Leu

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP3	66	43553	missense variant	<i>Org1_01695</i>	hypothetical protein	95C>A	Pro32Gln
SP3	66	61600	missense variant	<i>Org1_01489</i>	hypothetical protein	206G>C	Gly69Ala
SP3	66	76543	missense variant	<i>Org1_01939</i>	putative universal stress protein	247G>A	Val83Ile
SP3	66	84845	missense varian	<i>Org1_00143</i>	hypothetical protein	42G>A	Met14Ile
SP3	66	127787	missense variant	<i>pnp_1</i>	polyribonucleotide nucleotidyltransferase	247C>G	Arg83Gly
SP3	100	10208	missense variant	<i>Org1_00874</i>	hypothetical protein	82G>T	Ala28Ser
SP3	100	28077	missense variant	<i>atpE</i>	ATP synthase subunit c	62A>T	Asn21Ile
SP3	100	28378	missense variant	<i>Org1_00657</i>	hypothetical protein	500C>T	Thr167Ile
SP3	100	31303	missense variant	<i>walR</i>	transcriptional regulatory protein walR	319C>T	Arg107Cys
SP3	100	33408	missense variant	<i>pcrA</i>	ATP-dependent DNA helicase <i>PcrA</i>	761A>G	Asp254Gly

Strains	Day of passage	Mutation position	Mutation type	Mutated gene	Function	Base change	Amino acid change
SP3	100	34705	missense variant	<i>ybiV</i>	sugar phosphatase YbiV	527C>T	Ser176Leu
SP3	100	43553	missense variant	<i>Org1_01695</i>	hypothetical protein	95C>A	Pro32Gln
SP3	100	52557	frameshift variant	<i>sasA</i>	Adaptive-response sensory-kinase SasA	1277delA	Asn426fs
SP3	100	76543	missense variant	<i>Org1_01939</i>	putative universal stress protein	247G>A	Val83Ile
SP3	100	84845	missense variant	<i>Org1_00143</i>	hypothetical protein	42G>A	Met14Ile
SP3	100	93138	missense variant	<i>tcaR</i>	HTH-type transcriptional regulator <i>TcaR</i>	200C>T	Ala67Val
SP3	100	127787	missense variant	<i>pnp_1</i>	polyribonucleotide nucleotidyltransferase	247C>G	Arg83Gly
SP3	100	159526	stop gained, disruptive inframe deletion	<i>glpK</i>	glycerol kinase	792_863delTGGA ACTGGTGGCTTCATGTT AATGAATACAGGT-	Tyr264_Thr288delinsTer

IBG, isopropoxy benzene guanidine; SP1, SP2 and SP3 indicate three independent *S. aureus* 29213 cultures