Supporting Information for:

Bicarbonate alters bacterial susceptibility to antibiotics by targeting the proton motive force

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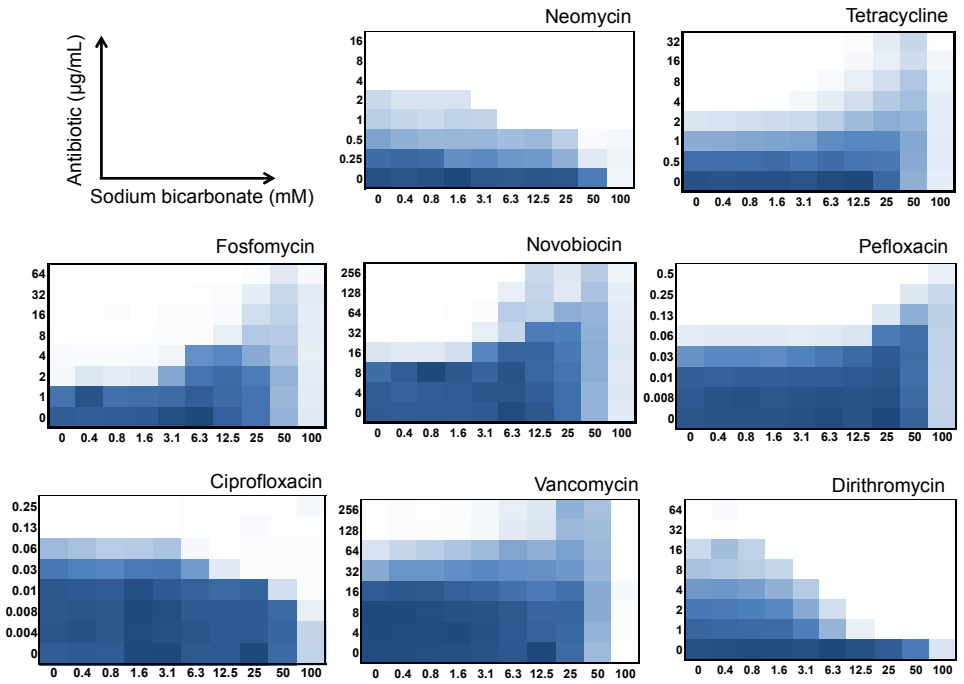


Figure S1. Microdilution checkerboard analyses for antibiotics in combination with sodium bicarbonate against *E. coli*. Shown are representative antibiotics whose activity was altered in the presence of 25 mM sodium bicarbonate. The extent of inhibition is shown as a heat plot, such that the darkest blue color represents full bacterial growth.

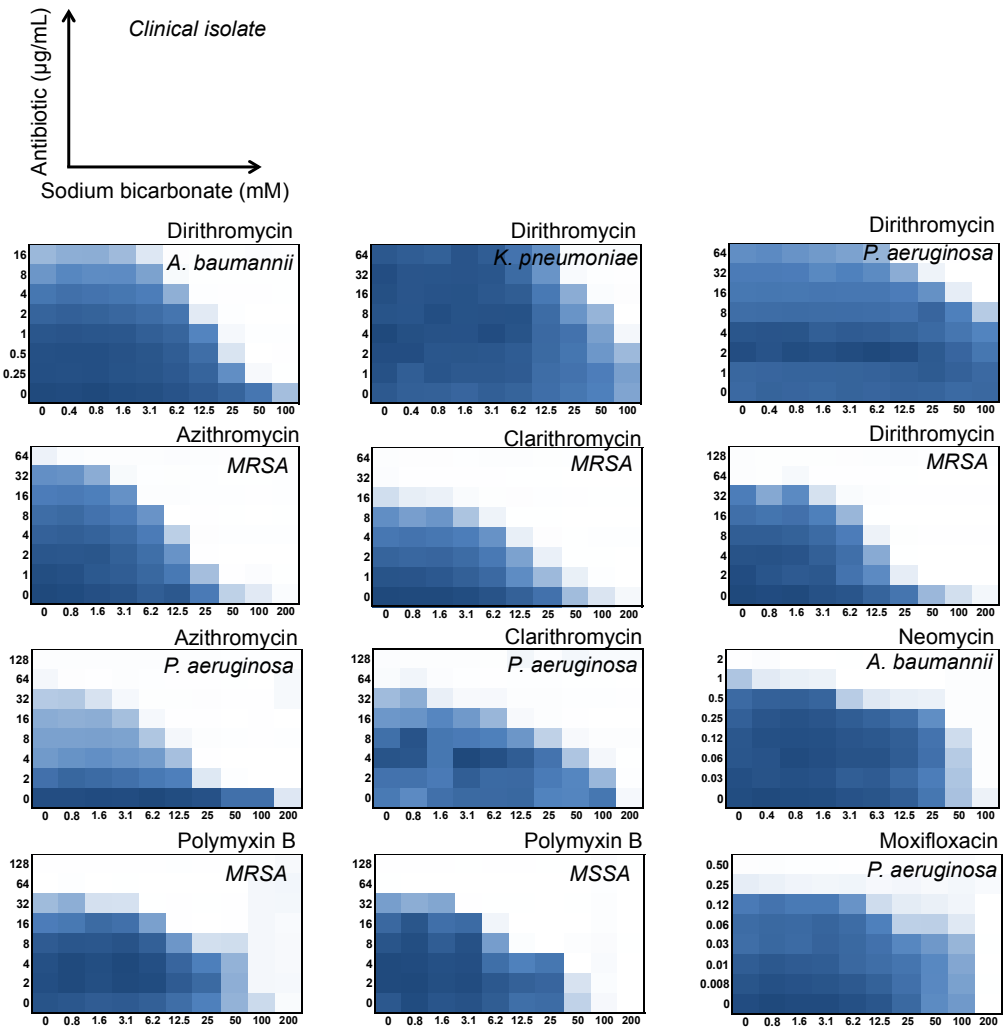


Figure S2. Combination of various antibiotics and sodium bicarbonate against multi-drug resistant clinical isolates, as listed in the checkerboard.

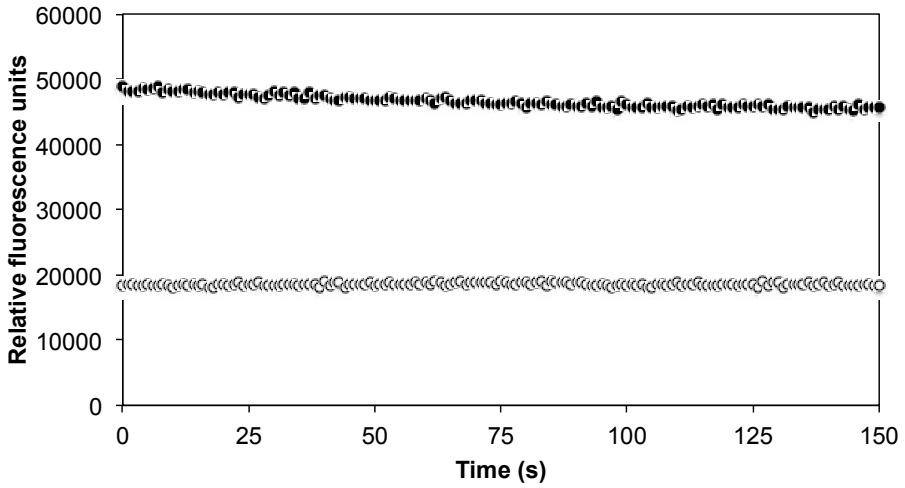


Figure S3. Uptake of 3,3'-Dipropylthiacarbocyanine iodide, a membrane-potential sensitive dye. *S. aureus* cells were grown to exponential phase in the absence (untreated) or presence of 25 mM sodium bicarbonate (treated), washed and loaded with 1 μM $DiSC_3(5)$. *S. aureus* treated with 25 mM sodium bicarbonate exhibited an increased uptake in the levels of $DiSC_3(5)$ as measured by fluorescence (black circles) and compared to uptake levels of untreated cells (white circles). Uptake and fluorescence was stable over time as shown in the graph.

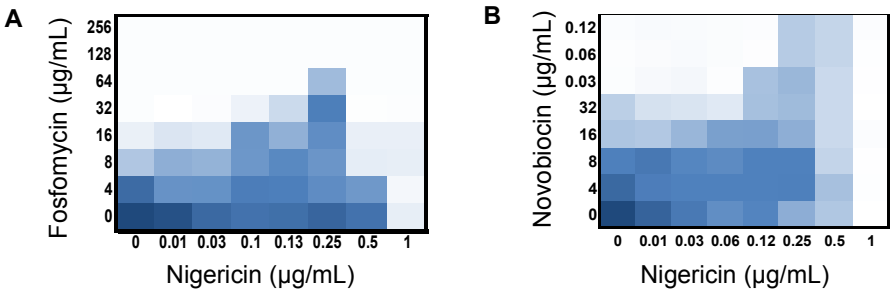


Figure S4. Combination of nigericin, a protonophore, with (A) fosfomycin or (B) novobiocin leads to antagonistic interactions against *S. aureus* (sensitive to nigericin). Shown are microdilution checkerboard analyses, where the extent of inhibition is shown as a heat plot, such that the darkest blue color represents full bacterial growth.

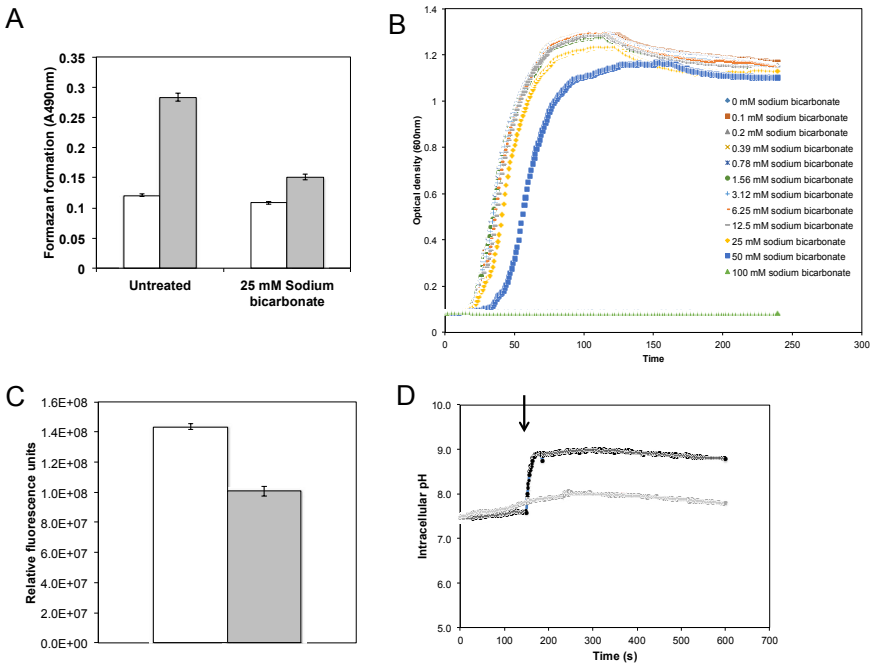


Figure S5. Sodium bicarbonate affects the proton motive force of bacteria. (A) Sodium bicarbonate inhibits cellular respiration. Shown is the effect of 25mM sodium bicarbonate on the reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to INT-formazan. Open bars indicate the formation of formazan at t=0 as read at 490 nm. Grey bars represent the formation of formazan following 60 min incubation. (B) Growth curve of *E. coli* grown in the presence of varying concentrations of sodium bicarbonate. Growth curve was performed in a microtiter plate and optical density read every 10 mins in a Tecan infinite M1000 Pro with shaking intervals before readings. (C) Effect of 25 mM sodium bicarbonate on intracellular ATP levels, measured by a luciferin-luciferase bioluminescence assay. Shown is the relative fluorescence units for untreated *S. aureus* cells (white bar) and for 25 mM bicarbonate treated *S. aureus* cells (grey). (D) Changes in intracellular pH upon treatment with bicarbonate. *S. aureus* cells were loaded with the pH sensitive dye BCECF-AM and were washed and resuspended in PBS. Following baseline readings, PBS (grey circles) or 25 mM sodium bicarbonate (black circles) were added at the arrow and fluorescence measured over time. A standard curve for intracellular pH calibration was used to calculate intracellular pH.

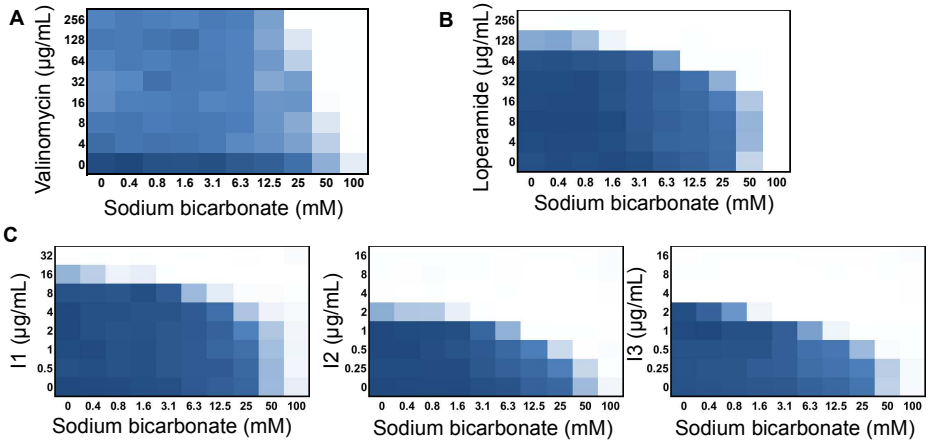


Figure S6. Microdilution checkerboard analyses for sodium bicarbonate in combination with molecules shown to dissipate ΔΨ; (A) valinomycin in *S. aureus* (B) loperamide in *E. coli* and (C) moleucles I1-3 [1] in *S. aureus*. All checkerboards display synergistic interactions.

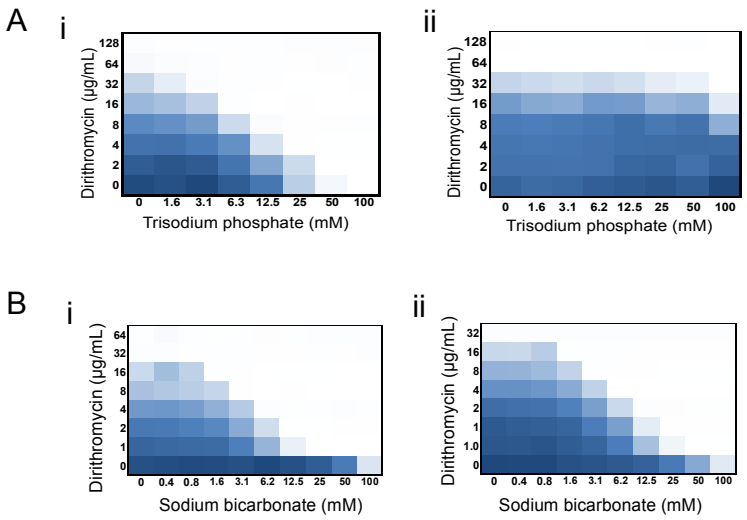


Figure S7. Bicarbonate alters ΔpH. (A) Effect of pH-adjusting media on the combination of dirithromycin with trisodium phosphate. Shown are microdilution checkerboard analyses for dirithromycin and trisodium phosphate when (i) pH is not adjusted to 7.2 (pH ~10) and when (ii) pH of the medium is adjusted to 7.2. (B) Growth inhibition by dirithromycin in combination with sodium bicarbonate. Bacterial strains were *E. coli* (i) wild-type and (ii) Δ*ychM*.

Table S1. MIC of various antibiotics in MHB vs MHB + 25 mM sodium bicarbonate against *E. coli* and *S. aureus*.

	E. coli			*S. aureus*		
	MHB-	MHB+	Fold	MHB-	MHB+	Fold
Apramycin	32	8	4	16	32	0.5
Gentamycin	2	0.25	8	2	1	2
Kanamycin	4	1	4	8	4	2
Neomycin	1	0.25	4	2	0.5	4
Paromomycin	2	1	2	2	2	1
Spectinomycin	16	4	4	64	8	8
Chloramphenicol	8	8	1	8	8	1
Dirithromycin	128	4	32	4	0.5	8
Erythromycin	128	32	4	32	1	32
Doxycycline	1	4	-4	0.125	2	-16
Tetracycline	1	4	-4	0.5	2	-4
Linezolid	256	256	1	0.625	0.625	1
Bacitracin	>256	>256	1	32	64	-2
Fosfomycin	4	16	-4	16	64	-4
Fosmidomycin	16	32	-2	>64	>64	1
Ampicillin	16	32	-2	1	8	-8
Amoxicillin	8	16	-2	0.25	1	-4
Cloxacillin	256	256	1	0.031	0.5	-16
Piperacillin	2	1	2	0.25	1	-4
Oxacillin	256	>256	-2	0.0625	1	-16
Ceftriaxone	0.625	0.625	1	4	16	-4
Cefoperazone	0.125	0.125	1	0.25	4	-16
Vancomycin	256	256	1	1	4	-4
Polymyxin B	0.25	0.5	-2	256	32	8
Ciprofloxacin	0.0625	0.0156	4	0.5	1	-2
Besifloxacin	0.25	0.0313	8	0.125	0.25	-2
Enoxacin	0.25	0.125	2	1	2	-2
Nalidixic acid	2	8	-4	>8	32	-4
Norfloxacin	0.125	0.0625	2	0.5	1	-2
Levofloxacin	0.0313	0.0313	1	0.5	1	-2
Moxifloxacin	0.0313	0.0156	2	0.125	0.0625	2
Pefloxacin	0.125	0.5	-4	0.5	2	-4
Novobiocin	32	>256	-8	0.031	0.5	-16
Rifampicin	32	64	-2	0.0078	0.031	-4

Table S2. Structural formula and physicochemical properties of fluoroquinolones. Listed are the pKa values for the acidic and basic functions of the fluoroquinolones, generated from ChemAxon, a physico-chemical property predictor.

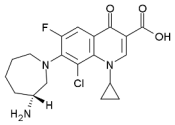
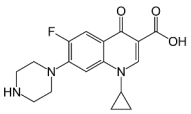
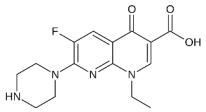
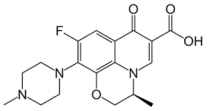
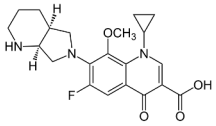
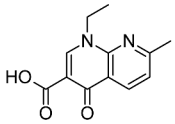
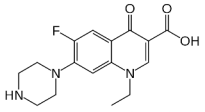
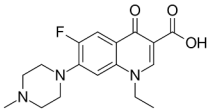
	Structure	pKa (Strongest Acidic)	pKa (Strongest Basic)
Besifloxacin		5.64	9.67
Ciprofloxacin		5.76	8.68
Enoxacin		5.5	8.59
Levofloxacin		5.45	6.2
Moxifloxacin		5.69	9.42
Nalidixic acid		5.95	4.68
Norfloxacin		5.77	8.68
Pefloxacin		5.66	6.47

Table S3. Chemical genomics interactions. For the genetic enhancers (Keio collection[2]) of growth inhibition by 25 mM bicarbonate, strains were exposed to bicarbonate for 15 hours in cation-adjusted MHB broth, and a multiplicative approach was used to determine the sick or lethal effects on each strain. Shown here are the outliers from Figure 3A, alongside their gene products, as annotated from EcoCyc[3]. For the GFP promoter-fusion library[4], shown is the list of promoters from the GFP promoter-fusion library that demonstrated increased or decreased promoter activity in the presence of 25 mM bicarbonate. Activity was assessed using the pipeline of Zaslaver *et al*[4]. Shown here are the promoters from Figure 3B, alongside their gene products, as annotated from EcoCyc[3].

Genetic enhancers of growth inhibition by 25 mM bicarbonate.	
Deletion strain	**Gene description**
appX	small outer membrane protein
cyaA	adenylate cyclase
cydX	cytochrome bd I terminal oxidase - CydX subunit
degP	serine protease Do
dnaT	primosomal protein DnaT
dsbB	protein disulfide oxidoreductase
envC	EnvC divisome associated factor, activator of peptidoglycan hydrolases
fur	Fur transcriptional dual regulator
galE	UDP-glucose 4-epimerase
glnA	adenylyl-[glutamine synthetase], glutamine synthetase
hfq	RNA-binding protein that affects many cellular processes; homolog of mammalian Sm/Sm-like proteins
lpoB	outer membrane lipoprotein - activator of MrcB activity
mgrB	negative feedback regulator of the PhoQP system
nhaA	Na^+:H^+ antiporter NhaA
pgi	phosphoglucose isomerase
recB	RecB
rodZ	transmembrane component of cytoskeleton
rplA	50S ribosomal subunit protein L1
rpmF	50S ribosomal subunit protein L32
rpoS	RNA polymerase, sigma S (sigma 38) factor
rpsT	30S ribosomal subunit protein S20
rsgA	ribosome small subunit-dependent GTPase A
sapA	periplasmic binding protein SapA of predicted ABC transporter
treA	periplasmic trehalase
ubiF	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone hydroxylase
ubiH	2-octaprenyl-6-methoxyphenol hydroxylase
ybbY	putative transport protein, nucleobase:cation symporter-2 (NCS2) family
ybcO	DLP12 prophage; predicted protein
yciB	inner membrane protein

Increased promoter activity	
Name	**Product**
ais	Predicted lipopolysaccharide core heptose(II)-phosphate phosphatase
alsB	D-allose ABC transporter - periplasmic binding protein
asd	Aspartate semialdehyde dehydrogenase
cspI	Qin prophage; cold shock protein
dinG	ATP-dependent helicase

dmlA	D-malate / 3-isopropylmalate dehydrogenase (decarboxylating)
dusA	tRNA-dihydrouridine synthase A
dusB	tRNA-dihydrouridine synthase B
entD	Phosphopantetheinyl transferase
erpA	Essential respiratory protein A
fetA	ABC transporter with a role in iron homeostasis - ATP-binding subunit
glyU	tRNA-glyU
htrL	Involved in lipopolysaccharide biosynthesis
iscR	IscR DNA-binding transcriptional dual regulator
kefF	Regulator of KefC-mediated potassium transport and quinone oxidoreductase
lpxC	UDP-3-O-acyl-N-acetylglucosamine deacetylase
mltC	Membrane-bound lytic murein transglycosylase C
murJ	Lipid II flippase
mutY	Adenine glycosylase; G.C --> T.A transversions
nhaA	Na+:H+ antiporter NhaA
potF	Putrescine ABC transporter - periplasmic binding protein
rcsC	RcsC sensory histidine kinase - asp875 phosphorylated
rfaH	RfaH transcriptional antiterminator
rplN	50S ribosomal subunit protein L14
rplY	50S ribosomal subunit protein L25
rpsJ	30S ribosomal subunit protein S10
rpsM	30S ribosomal subunit protein S13
rpsO	30S ribosomal subunit protein S15
rpsP	30S ribosomal subunit protein S16
rsfS	Ribosomal silencing factor
smpB	Small protein B
sppA	Protease IV, a signal peptide peptidase
tig	Chaperone protein Tig; trigger factor
trxA	Oxidized thioredoxin, thioredoxin 1
ttcA	tRNA C32 thiolase
waaZ	Protein involved in KdoIII attachment during lipopolysaccharide core biosynthesis
yacG	DNA gyrase inhibitor YacG
ybaA	Conserved protein
ybaB	Conserved DNA-binding protein
ybfE	LexA-regulated protein
ycgM	Predicted isomerase/hydrolase
ydeA	Arabinose exporter
yeiE	LYSR-type transcriptional regulator
yejL	Conserved protein
yidH	Conserved inner membrane protein
yjdI	Conserved protein
yncE	Conserved protein
yphG	Conserved protein
zraP	Zinc responsive, periplasmic protein with chaperone activity

Decreased promoter activity	
Name	**Product**
aspU	tRNA-aspU
bioB	Biotin synthase
deaD	DeaD, DEAD-box RNA helicase

fadE	Acyl-CoA dehydrogenase
folD	Bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/ 5,10-methylene-tetrahydrofolate cyclohydrolase
fpr	Flavodoxin-NADP+ reductase / ferredoxin-NADP+ reductase
ftp	Flavin transferase
ftsZ	Essential cell division protein FtsZ
gcvA	GcvA DNA-binding transcriptional dual regulator
glnA	Adenylyl-[glutamine synthetase], glutamine synthetase
glrK	GlrK sensory histidine kinase - phosphorylated, GlrK sensory histidine kinase
hemA	Glutamyl-tRNA reductase
hfq	RNA-binding protein that affects many cellular processes; homolog of mammalian Sm/Sm-like proteins
hofM	Protein involved in utilization of DNA as a carbon source
hscC	Hsc62, Hsp70 family chaperone, binds to RpoD and inhibits transcription
lysO	L-lysine exporter
metZ	TRNA-fMet1
pepQ	Xaa-Pro dipeptidase
polB	DNA polymerase II
prlF	PrlF antitoxin
radD	Predicted ATP-dependent helicase; implicated in DNA repair
rapA	RNA polymerase-binding ATPase and RNAP recycling factor
rhaD	Rhamnulose-1-phosphate aldolase
rpmI	50S ribosomal subunit protein L35
rrfG	rrfG 5S ribosomal RNA
rrlH	rrlH 23S ribosomal RNA
sbcB	Exonuclease I, 3' --> 5' specific; deoxyribophosphodiesterase
tcdA	tRNA threonylcarbamoyladenosine dehydratase
yahK	Aldehyde reductase, NADPH-dependent
ybiO	Mechanosensitive channel YbiO
ydbC	Predicted oxidoreductase, NAD(P)-binding
yegW	Predicted DNA-binding transcriptional regulator
ygbI	Predicted DNA-binding transcriptional regulator, DEOR-type
yiaT	Outer membrane protein YiaT
yieH	6-Phosphogluconate phosphatase
yjbF	Predicted lipoprotein
yjfY	Putative protein
ykgF	Predicted amino acid dehydrogenase with NAD(P)-binding domain and ferridoxin-like domain
ykgJ	Predicted ferredoxin
ynfC	YnfC lipoprotein
yodB	Predicted cytochrome
ypdA	YbdA sensory histidine kinase - his371 phosphorylated

Table S4. MIC of sodium bicarbonate against various pathogens.

Organism	MIC (mM)
Escherichia coli	100
Staphylococcus aureus	50-100
Klebsiella pneumoniae	100
Acinetobacter baumannii	50
Pseudomonas aeruginosa	>100
Enterococcus faecium	50-100

Table S5. MIC of various components of innate immunity in MHB vs MHB + 25 mM sodium bicarbonate against *S. aureus* (strain Newman).

Component	MIC (μg/mL) in MHB	MIC (μg/mL) in MHB + 25 mM bicarbonate
LL-37	128	32
Indolicidin	128	8
Bactenesin	128	0.5
Protegrin	32	4
Lysozyme	>256*	>256*
Bile salts	512	512
Hyaluronic acid	4	0.0625

**S. aureus* is intrinsically resistant to lysozyme

References

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