Antibiotic Resistance Increases with Local Temperature: Supplementary Information

a

Average min temp	Average temp	Negative latitude
Trimethoprim-Sulfamethoxazole	Trimethoprim-Sulfamethoxazole	Trimethoprim-Sulfamethoxazole
Amoxicillin	Amoxicillin	Amoxicillin
Tetracycline/Doxycycline	Tetracycline/Doxycycline	Tetracycline/Doxycycline
Ampicillin-Sulbactam	Ampicillin-Sulbactam	Ampicillin-Sulbactam
Gentamicin	Gentamicin	Gentamicin
Tobramycin	Tobramycin	Tobramycin
Levofloxacin	Levofloxacin	Levofloxacin
Cefepime	Cefepime	Cefepime
Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
Cephalexin	Cephalexin	Cephalexin
Piperacillin-Tazobactam	Piperacillin-Tazobactam	Piperacillin-Tazobactam
Ceftazidime	Ceftazidime	Ceftazidime
Ceftriaxone	Ceftriaxone	Ceftriaxone
Amoxicillin-Clavulanic acid	Amoxicillin-Clavulanic acid	Amoxicillin-Clavulanic acid
Nitrofurantoin	Nitrofurantoin	Nitrofurantoin
Amikacin	Amikacin	Amikacin
Aztreonam	Aztreonam	Aztreonam
Imipenem	Imipenem	Imipenem
Meropenem	Meropenem	Meropenem
Cefuroxime	Cefuroxime	Cefuroxime
Ertapenem	Ertapenem	Ertapenem

b

Average min temp	Average temp	Negative latitude
Gentamicin	Gentamicin	Gentamicin
Ceftazidime	Ceftazidime	Ceftazidime
Trimethoprim-Sulfamethoxazole	Trimethoprim-Sulfamethoxazole	Trimethoprim-Sulfamethoxazole
Ampicillin-Sulbactam	Ampicillin-Sulbactam	Ampicillin-Sulbactam
Levofloxacin	Levofloxacin	Levofloxacin
Tobramycin	Tobramycin	Tobramycin
Cephalexin	Cephalexin	Cephalexin
Piperacillin-Tazobactam	Piperacillin-Tazobactam	Piperacillin-Tazobactam
Ceftriaxone	Ceftriaxone	Ceftriaxone
Tetracycline/Doxycycline	Tetracycline/Doxycycline	Tetracycline/Doxycycline
Cefepime	Cefepime	Cefepime
Aztreonam	Aztreonam	Aztreonam
Nitrofurantoin	Nitrofurantoin	Nitrofurantoin
Amoxicillin-Clavulanic acid	Amoxicillin-Clavulanic acid	Amoxicillin-Clavulanic acid
Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
Imipenem	Imipenem	Imipenem
Ertapenem	Ertapenem	Ertapenem
Amikacin	Amikacin	Amikacin
Meropenem	Meropenem	Meropenem
Cefuroxime	Cefuroxime	Cefuroxime

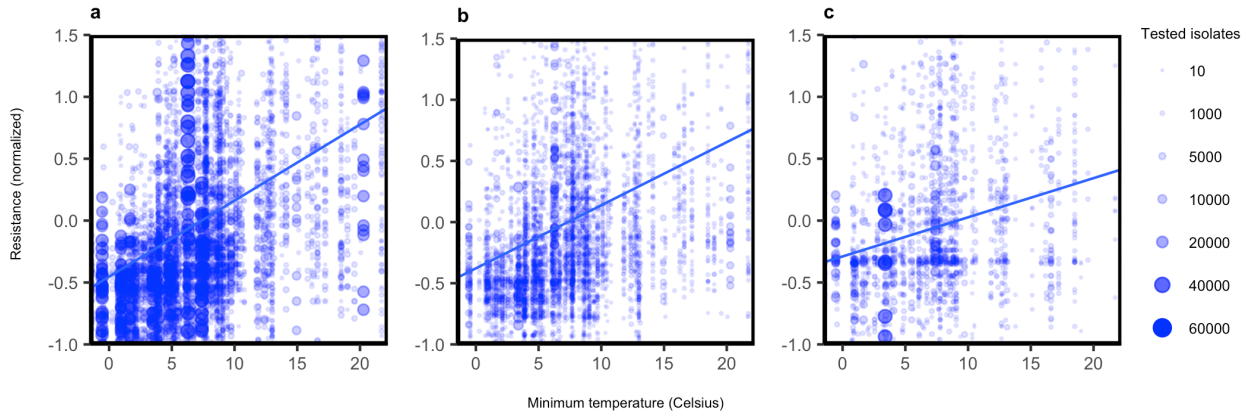
Correlation with percent resistance

0.6
0.4
0.2
0.0

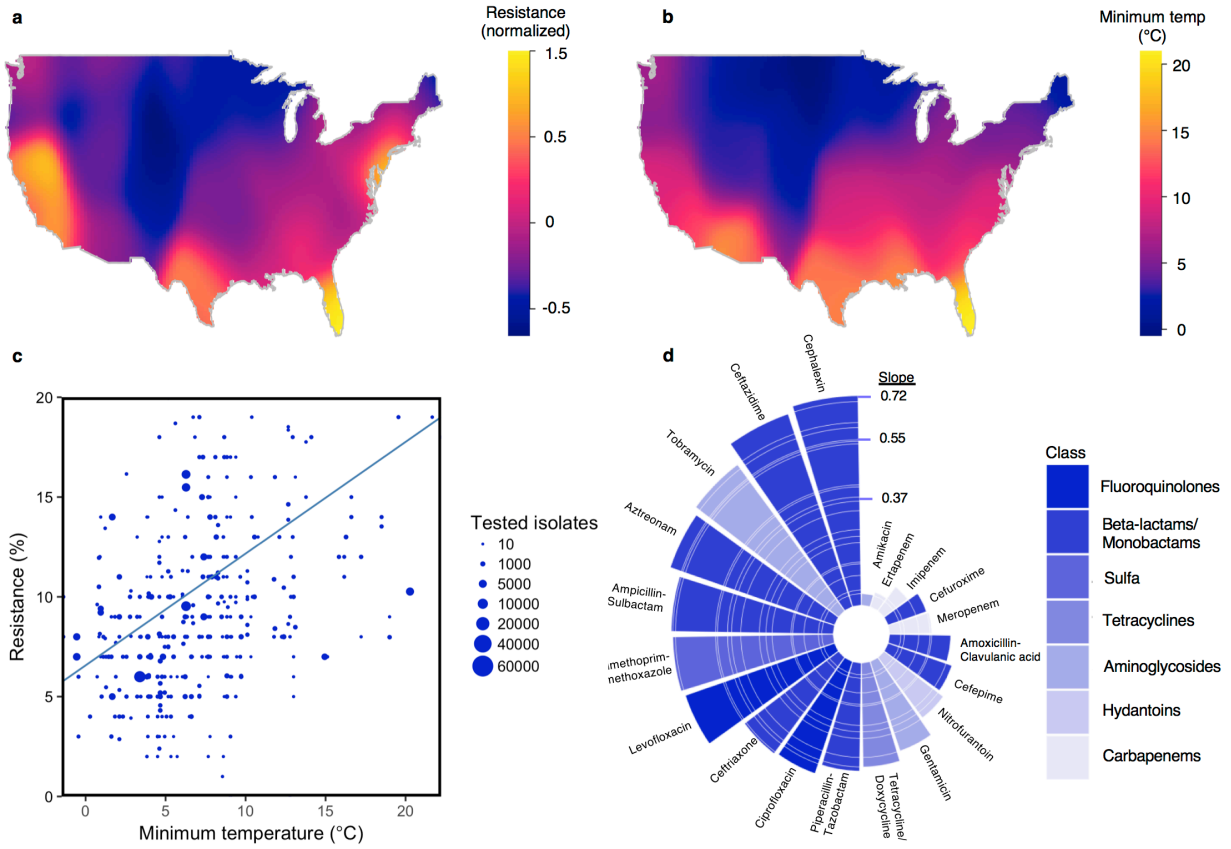
c

Average min temp	Average temp	Negative latitude
Quinupristin/Dalfupristin	Quinupristin/Dalfupristin	Quinupristin/Dalfupristin
Erythromycin	Erythromycin	Erythromycin
Trimethoprim-Sulfamethoxazole	Trimethoprim-Sulfamethoxazole	Trimethoprim-Sulfamethoxazole
Cloxacillin	Cloxacillin	Cloxacillin
Nitrofurantoin	Nitrofurantoin	Nitrofurantoin
Cephalexin	Cephalexin	Cephalexin
Levofloxacin	Levofloxacin	Levofloxacin
Rifampin	Rifampin	Rifampin
Tetracycline/Doxycycline	Tetracycline/Doxycycline	Tetracycline/Doxycycline
Vancomycin	Vancomycin	Vancomycin
Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
Nafcillin	Nafcillin	Nafcillin
Clindamycin	Clindamycin	Clindamycin
Daptomycin	Daptomycin	Daptomycin
Penicillin	Penicillin	Penicillin
Linezolid	Linezolid	Linezolid
Moxifloxacin	Moxifloxacin	Moxifloxacin

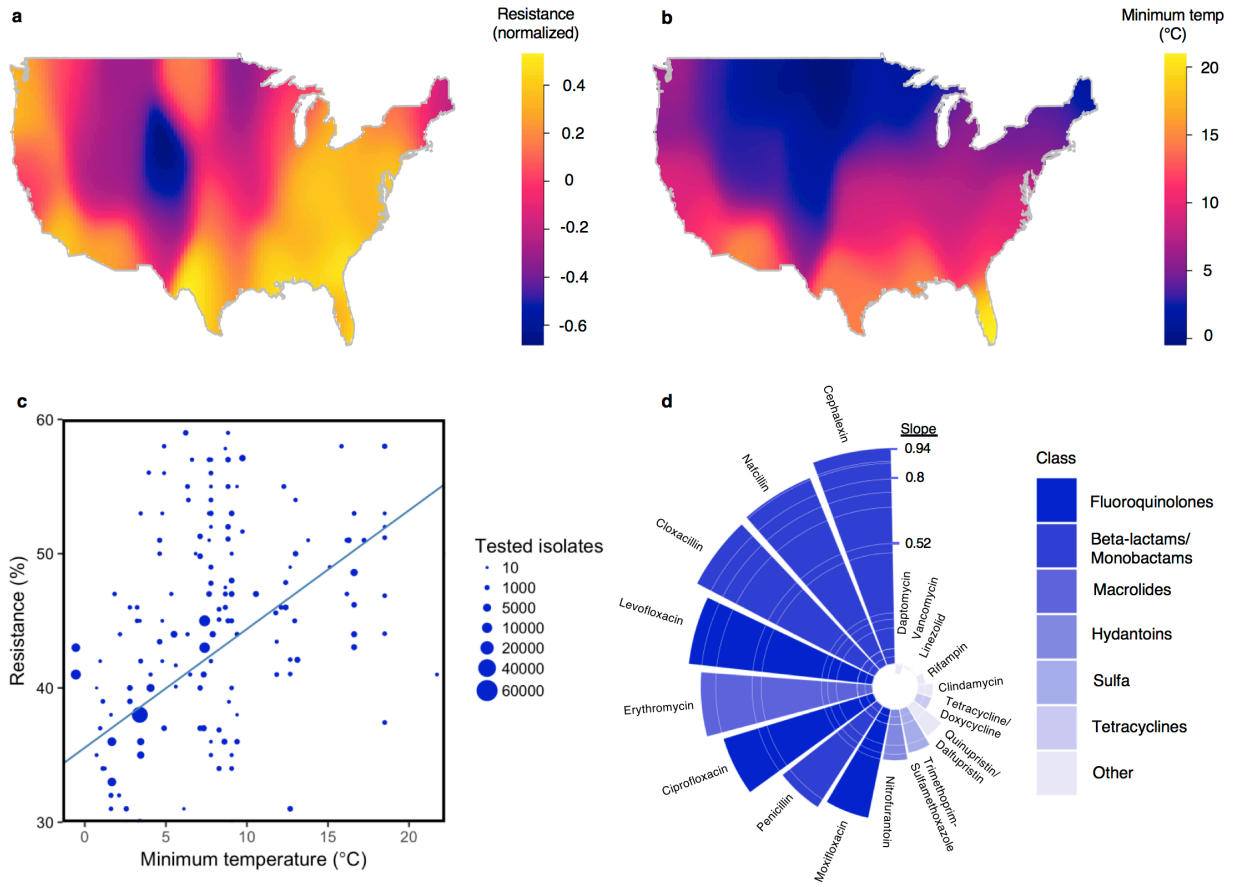
Supplementary Figure 1 | Correlation between climate and latitude variables with antibiotic resistance. Correlation of predictor (30-year mean minimum temperature, 30-year mean temperature, latitude) with antibiotic resistance (%) by pathogen: (A) *E. coli*; (B) *K. pneumoniae*; and (C) *S. aureus*.



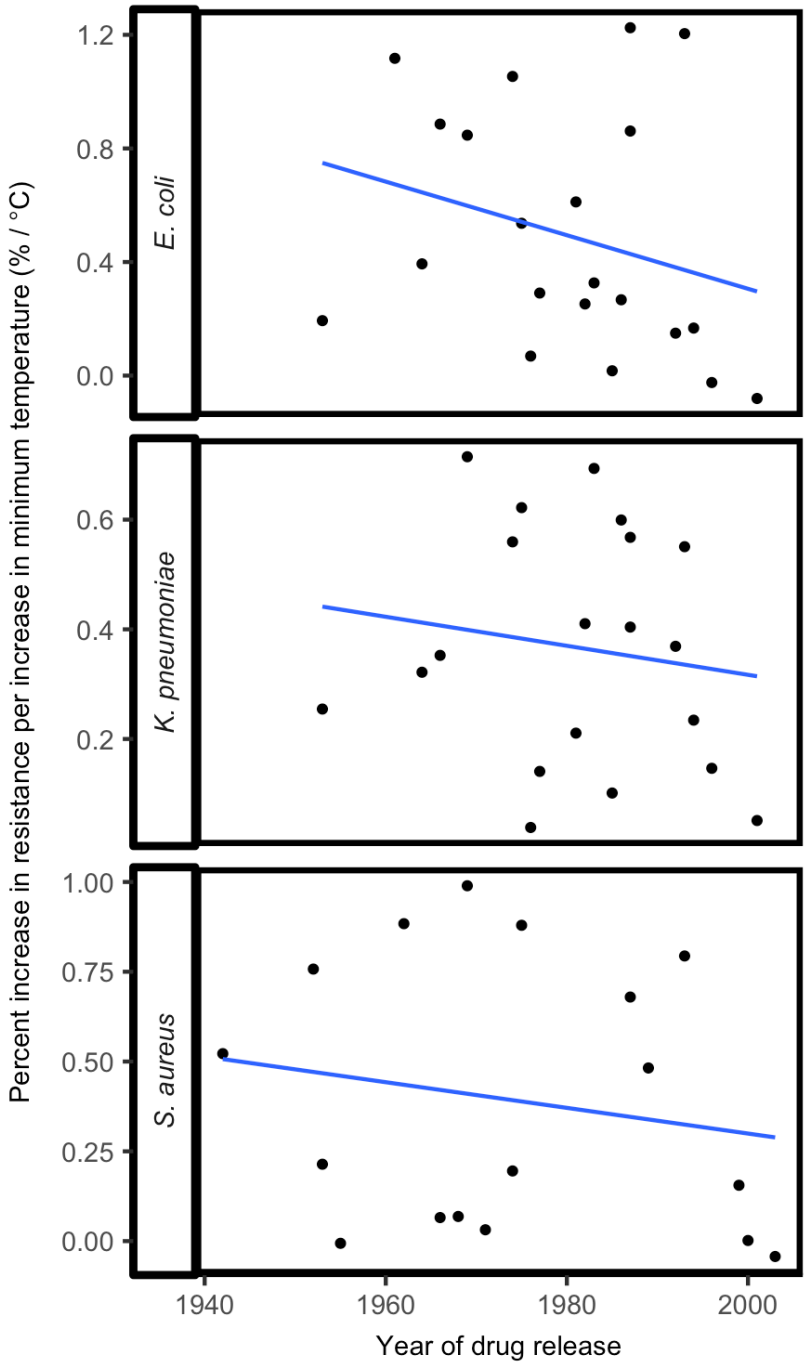
Supplementary Figure 2 | Association between minimum temperature and antibiotic resistance. Scatter plots of mean normalized antibiotic resistance versus minimum temperature (^{o}C) for all tested antibiotics, by pathogen: (A) *E. coli*; (B) *K. pneumoniae*; and (C) *S. aureus*. Unadjusted weighted linear trend lines are shown in blue.



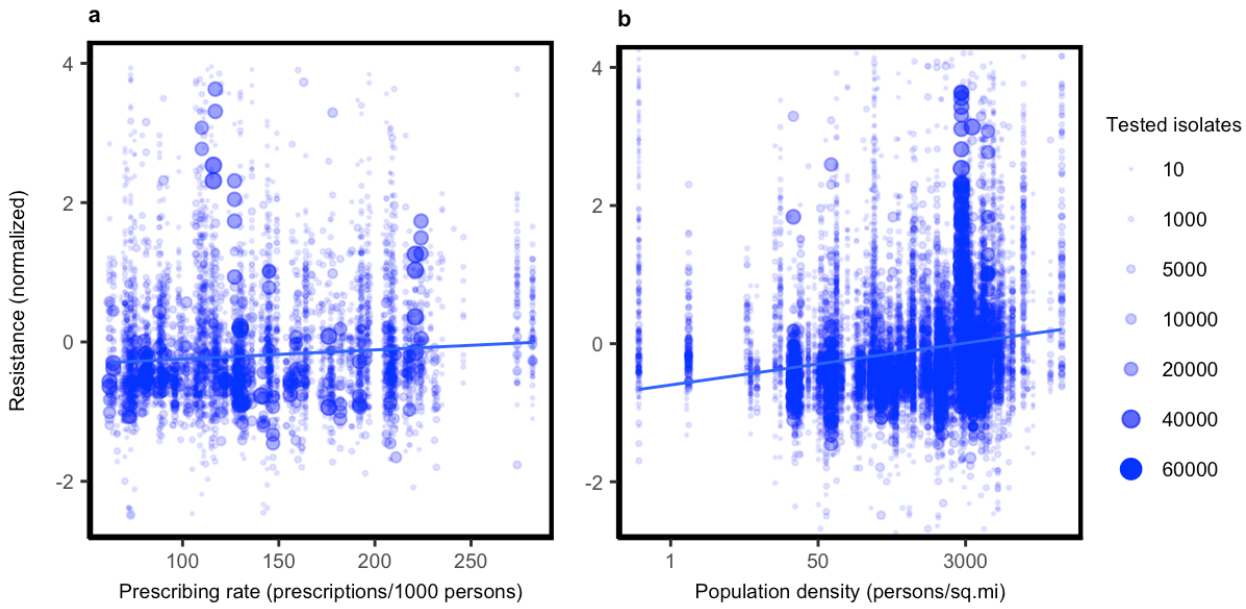
Supplementary Figure 3 | Antibiotic resistance and minimum temperature (*K. pneumoniae*). (A) A heatmap of mean normalized antibiotic resistance for *K. pneumoniae* for all antibiotics across the United States. (B) A heatmap of 30-year average minimum temperature (^{o}C) across the United States. (C) A scatter plot of antibiotic resistance versus minimum temperature for *K. pneumoniae* and trimethoprim-sulfamethoxazole. Unadjusted weighted linear trend line is shown in blue. (D) Slope of unadjusted relationship (% Resistance/^{o}C) between minimum temperature and antibiotic resistance by antibiotic for *K. pneumoniae.* Antibiotic class coded by color shading.



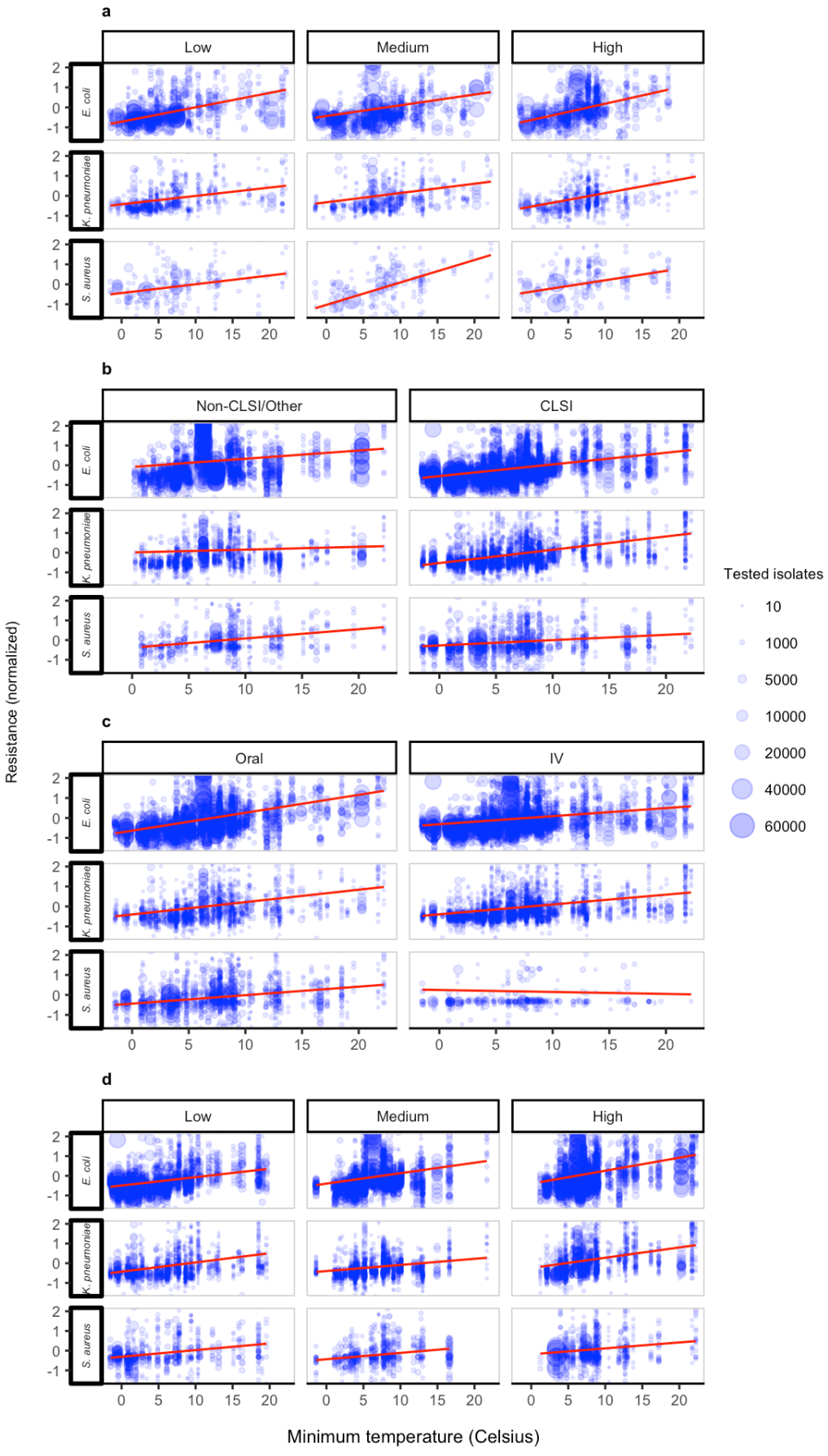
Supplementary Figure 4 | Antibiotic resistance and minimum temperature (*S. aureus*). (A) A heatmap of mean normalized antibiotic resistance for *S. aureus* for all antibiotics across the United States. (B) A heatmap of 30-year average minimum temperature (^{o}C) across the United States. (C) A scatter plot of antibiotic resistance versus minimum temperature for *S. aureus* and cloxacillin. Unadjusted weighted linear trend line is shown in blue. (D) Slope of unadjusted relationship (% Resistance/^{o}C) between minimum temperature and antibiotic resistance by antibiotic for *S. aureus.* Antibiotic class coded by color shading.



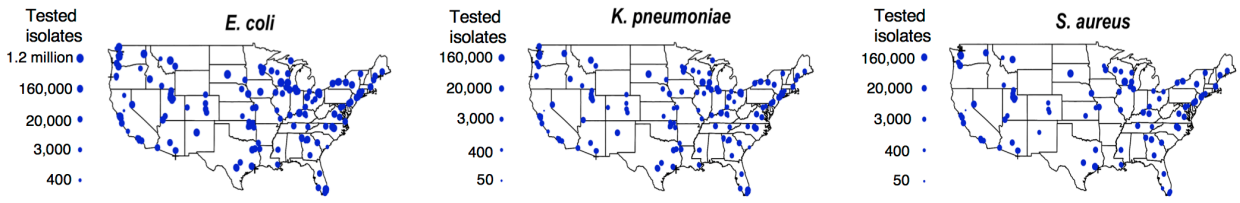
Supplementary Figure 5 | Antibiotic specific association between minimum temperature and antibiotic resistance versus approximate antibiotic introduction time in the United States[1].



Supplementary Figure 6 | Additional important predictors of antibiotic resistance, population density and prescribing rate. Scatter plots of the association between mean normalized antibiotic resistance with (A) annual prescription rate (prescriptions per 1000 persons); and (B) population density (persons/mi^2), in *E. coli*, *K. pneumoniae*, and *S. aureus*. Data point size corresponds to number of isolates. Unadjusted weighted linear trend lines are shown in blue.


a
Low
Medium
High
b
Non-CLSI/Other
CLSI
c
Oral
IV
d
E. coli
K. pneumoniae
S. aureus
Resistance (normalized)
Minimum temperature (Celsius)
Tested isolates
10
1000
5000
10000
20000
40000
60000

Supplementary Figure 7 | Evaluating relationship of minimum temperature and antibiotic resistance across other covariates. Scatter plots of mean normalized antibiotic resistance (%) versus minimum temperature (^{o}C) by: (A) tertile of prescription rate (annual prescriptions/1000 persons); (B) reported laboratory standard (CLSI vs. other/not reported); (C) oral/IV formulation; and (D) tertile of population density (persons/mi^2). Unadjusted weighted linear trend lines are shown in red.



Supplementary Figure 8 | Distribution of antibiotic resistance data across the United States. A map of the United States showing locations of antibiotic resistance indices used as data points in this analysis, as a function of number of isolate tests (data point size), by pathogen (A) *E. coli*, (B) *K. pneumoniae*, and (C) *S. aureus*.

Supplementary Table 1 | Antibiotic and organism specific adjusted estimates of association between minimum temperature and antibiotic resistance.

Bacteria	Drug	Minimum temp	P-Value
E. coli	Amoxicillin	0.98	<0.0001
	Amoxicillin-Clavulanic acid	0.31	0.08
	Ampicillin-Sulbactam	0.64	<0.0001
	Cefepime	0.06	0.22
	Ceftazidime	0.26	0.0005
	Ceftriaxone	0.15	0.13
	Cefuroxime	0.19	0.40
	Cephalexin	0.61	<0.0001
	Ciprofloxacin	0.56	0.12
	Levofloxacin	0.67	0.0018
	Piperacillin-Tazobactam	0.14	<0.0001
K. pneumoniae	Amoxicillin-Clavulanic acid	-0.12	0.11
	Ampicillin-Sulbactam	0.36	<0.0001
	Cefepime	0.04	0.17
	Ceftazidime	0.45	<0.0001
	Ceftriaxone	0.19	<0.0001
	Cefuroxime	0.02	0.83
	Cephalexin	0.52	<0.0001
	Ciprofloxacin	0.11	0.05
	Levofloxacin	0.28	<0.0001
	Piperacillin-Tazobactam	0.23	<0.0001
S. aureus	Cephalexin	0.51	0.10
	Ciprofloxacin	0.29	0.0001
	Cloxacillin	0.58	<0.0001
	Erythromycin	0.54	<0.0001
	Levofloxacin	0.39	0.18
	Moxifloxacin	0.89	0.16
	Nafcillin	0.84	0.10
	Penicillin	-0.07	0.87

Sub-analysis of selected effect estimates and p-values for the association between minimum temperature and antibiotic resistance from full adjusted multivariable models restricted to specific antibiotic susceptibilities (for those antibiotics with available prescribing data). Individual models allow for different effects (modification) by antibiotic type and organism, but are subject to reduced sample size/power.

Supplementary Table 2 | Model predictor variable correlations.

Correlation Matrix

			E. coli			
	T min	Prescription rate	Outpatient	Lab standard	Population density	Median income
T min	1.000					
Prescription rate	0.008	1.000				
Outpatient	-0.123	0.042	1.000			
Lab standard	-0.048	0.009	-0.025	1.000		
Population density	0.237	-0.096	0.040	-0.060	1.000	
Median income	0.081	-0.017	0.028	-0.086	0.443	1.000
			Klebsiella			
	T min	Prescription rate	Outpatient	Lab standard	Population density	Median income
T min	1.000					
Prescription rate	0.005	1.000				
Outpatient	-0.119	0.042	1.000			
Lab standard	-0.033	0.015	-0.014	1.000		
Population density	0.224	-0.116	0.047	-0.057	1.000	
Median income	0.073	-0.033	0.046	-0.095	0.451	1.000
			S. aureus			
	T min	Prescription rate	Outpatient	Lab standard	Population density	Median income
T min	1.000					
Prescription rate	0.029	1.000				
Outpatient	-0.226	0.018	1.000			
Lab standard	-0.105	0.036	-0.070	1.000		
Population density	0.188	-0.072	0.061	-0.092	1.000	
Median income	0.046	-0.094	-0.061	-0.156	0.500	1.000

Variance inflation factor (VIF)

Bacteria	T min	Prescription rate	Outpatient	Lab standard	Population density	Median income
E. coli	1.217	1.012	1.020	1.114	1.273	1.239
Klebsiella	1.195	1.011	1.029	1.108	1.392	1.422
S. aureus	1.274	1.024	1.124	1.253	1.473	1.450

Supplementary Table 3 | IV and Oral antibiotic classification.

E. coli		*K. pneumoniae*		*S. aureus*	
Amoxicillin	Oral	Amoxicillin-Clavulanic acid	Oral	Cephalexin	Oral
Amoxicillin-Clavulanic acid	Oral	Cefuroxime	Oral	Clindamycin	Oral
Cefuroxime	Oral	Cephalexin	Oral	Cloxacillin	Oral
Cephalexin	Oral	Ciprofloxacin	Oral	Erythromycin	Oral
Ciprofloxacin	Oral	Levofloxacin	Oral	Levofloxacin	Oral
Levofloxacin	Oral	Tetracycline/Doxycycline	Oral	Linezolid	Oral
Nitrofurantoin	Oral	Trimethoprim-Sulfamethoxazole	Oral	Moxifloxacin	Oral
Tetracycline/Doxycycline	Oral	Amikacin	IV	Penicillin	Oral
Trimethoprim-Sulfamethoxazole	Oral	Ampicillin-Sulbactam	IV	Rifampin	Oral
Amikacin	IV	Aztreonam	IV	Tetracycline/Doxycycline	Oral
Ampicillin-Sulbactam	IV	Cefepime	IV	Trimethoprim-Sulfamethoxazole	Oral
Aztreonam	IV	Ceftazidime	IV	Daptomycin	IV
Cefepime	IV	Ceftriaxone	IV	Nafcillin	IV
Ceftazidime	IV	Ertapenem	IV	Quinupristin/Dalfupristin	IV
Ceftriaxone	IV	Gentamicin	IV	Vancomycin	IV
Ertapenem	IV	Imipenem	IV		
Gentamicin	IV	Meropenem	IV		
Imipenem	IV	Piperacillin-Tazobactam	IV		
Meropenem	IV	Tobramycin	IV		
Piperacillin-Tazobactam	IV				
Tobramycin	IV				

Supplementary Table 4 | Additional sensitivity analyses.

Bacteria	Variable	Sensitivity A	Sensitivity B	Sensitivity C	Sensitivity D	Sensitivity E	Sensitivity F
E. coli	Minimum temp	0.44 *(<0.0001)*	0.47 *(<0.0001)*	0.34 *(<0.0001)*	0.41 *(<0.0001)*	0.38 *(<0.0001)*	
	Prescription rate	0.12 *(<0.0001)*	0.11 *(<0.0001)*	0.11 *(<0.0001)*	0.12 *(<0.0001)*	0.12 *(<0.0001)*	
	Outpatient	-2.99 *(0.09)*	-3.30 *(<0.001)*	-4.23 *(0.04)*	-2.98 *(0.07)*	-3.08 *(0.07)*	
	Lab standard	-3.82 *(0.09)*	-2.43 *(0.18)*	-5.49 *(0.07)*	-3.20 *(0.14)*	-3.13 *(0.16)*	
	Population density	0.00028 *(0.13)*	0.00030 *(0.06)*	0.00020 *(0.20)*	--	0.00015 *(0.28)*	
	Median income		--		0.00029 *(<0.001)*	0.00025 *(0.001)*	
	Blood		-3.93 *(0.04)*				
	Respiratory		2.95 *(0.29)*				
	Urine		-1.15 *(0.28)*				
	Other sterile		11.91 *(<0.001)*				
	Other nonsterile		-14.29 *(<0.0001)*				
Klebsiella	Minimum temp	0.23 *(<0.001)*	0.22 *(<0.0001)*	0.29 *(<0.0001)*	0.28 *(<0.0001)*	0.21 *(<0.0001)*	
	Prescription rate	0.04 *(<0.0001)*	0.03 *(<0.0001)*	0.02 *(<0.0001)*	0.03 *(<0.0001)*	0.03 *(<0.0001)*	
	Outpatient	-2.68 *(0.20)*	-2.75 *(0.13)*	-4.00 *(0.15)*	-2.55 *(0.19)*	-2.91 *0.15*	
	Lab standard	-0.40 *(0.76)*	-0.38 *(0.66)*	-0.43 *(0.74)*	-0.71 *(0.65)*	-0.34 *0.76*	
	Population density	0.00062 *(<0.0001)*	0.00060 *(<0.0001)*	0.00064 *(<0.0001)*	--	0.00058 *(<0.0001)*	
	Median income		--		0.00028 *(0.16)*	0.00004 *0.79*	
	Blood		-0.53 *(0.65)*				
	Respiratory		1.27 *(0.37)*				
	Urine		-1.86 *(0.27)*				
	Other sterile		-10.64 *(<0.0001)*				
	Other nonsterile		10.35 *(<0.0001)*				
S. aureus	Minimum temp	0.68 *(0.002)*	0.19 *(<0.0001)*	0.39 *(0.14)*	0.28 *(0.18)*	0.28 *(0.18)*	0.53 *(0.002)*
	Prescription rate	0.12 *(<0.0001)*	0.13 *(<0.0001)*	0.10 *(<0.001)*	0.13 *(<0.0001)*	0.13 *(<0.0001)*	0.17 *(<0.0001)*
	Outpatient	-7.93 *(<0.0001)*	-4.47 *(<0.001)*	-0.18 *(0.89)*	-3.92 *(0.006)*	-3.86 *(0.008)*	-6.22 *(<0.0001)*
	Lab standard	-1.38 *(0.55)*	-3.89 *(0.06)*	-9.50 *(0.05)*	-3.62 *(0.03)*	-3.65 *(0.05)*	-2.69 *(0.08)*
	Population density	0.00006 *(0.14)*	0.00000 *(0.97)*	-0.00013 *(0.44)*	--	-0.00003 *(0.84)*	0.00014 *(0.15)*
	Median income		--		0.00010 *(0.38)*	0.00020 *(0.33)*	
	Blood		-4.24 *(<0.001)*				
	Respiratory		2.78 0.22				
	Urine		8.59 *(<0.001)*				
	Other sterile		-17.63 *(<0.0001)*				
	Other nonsterile		19.80 *(<0.0001)*				

(A) restriction of the full adjusted model to only health systems, hospitals, or laboratories (excluding surveillance bodies); (B) full adjusted model with restriction of the dataset to only locations with multiple years represented within the database; (C) full adjusted model with addition of binary predictors for isolate types; (D) full adjusted model with substitution of population density variable with median income; (E) full adjusted model with addition of median income; and (F) full adjusted model for *S. aureus* with cephalexin and nafcillin excluded. P-values are shown in parentheses (). All analyses were restricted to subset of antibiotic susceptibilities for dates and classes of antibiotics corresponding to available prescribing rate data.

Supplementary References

1. IUPAC, Fischer, J. & Robin Ganellin, C. *Analogue-based Drug Discovery*. (John Wiley & Sons, 2006).