The introduction of beta-lactam antibiotics in 1944 began with penicillin and was a triumph of modern medicine. Among antibiotics, beta-lactams were particularly regarded as magic bullets, because of their reliability in treating bacterial infections, and their minimal side effects on humans. However, shortly after their introduction, bacteria started expressing enzymes from mobile plasmids that could hydrolyze and inactivate the beta-lactams.

In 1963, the TEM beta-lactamase emerged among gram negative bacteria, and it rapidly increased in frequency to become the most frequent beta-lactamase in most pathogenic gram negative populations. TEM stands for Temoneira, the name of the patient from whom the enzyme was first isolated. TEM beta-lactamases have been found in Escherichia coli, *Klebsiella pneumoniae* and other gram-negative bacteria. TEM-1 is considered the wild-type. The length of TEM-1 is 287, i.e., TEM-1 can be represented as a sequence of 287 letters in the 20-letter alphabet corresponding to the amino acids. Over 170 TEM variants have been found clinically, where 41 are single mutants, i.e., they have exactly one amino acid substitution, and the majority (90 %) has at most 4 amino acid substitutions.

The TEM-50 variant contains four mutations. We created all 16 possible variations of those mutations using site directed mutagenesis.

Number of Substitutions	**Binary Allele Code**	**Variants with mutations found in *bla*$_{\text{TEM-50}}$**
0	0000	No Mutations (TEM-1)
1	1000	M69L

		(TEM-33)
1	0100	E104K (TEM-17)
1	0010	G238S (TEM-19)
1	0001	N276D (TEM-84)
2	1100	M69L E104K (Not identified)
2	1010	M69L G238S (Not identified)
2	1001	M69L N276D (TEM-35)
2	0110	E104K G238S (TEM-15)
2	0101	E104K N276D (Not identified)
2	0011	G238S N276D (Not identified)
3	1110	M69L

		E104K G238S (Not identified)
3	1101	M69L E104K N276D (Not Identified)
3	1011	M69L G238S N276D (Not identified)
3	0111	E104K G238S N276D (Not identified)
4	1111	M69L E104K G238S N276D (TEM-50)

We determined the growth rate of the strains expressing each variant in one of 14 antibiotics including: AM (amoxicillin), AMC (amoxicillin clavulate), AMP (Ampicillin), CAZ (ceftazidime), CEC (ceflacor), CPD (cefpodoxime), CPR (Cefprozil), CRO (cefuroxime), CTT (cefotetan), CTX (cefotaxime), FEP (cefepime), SAM (Ampicillin sulbactam), TZP (pipercillin tazobactam) and ZOX (ceftizoxime).

There were 12 replicates for each sample in each antibiotic.

We computed the mean and variance of each and computed significance between adjacent variants that differ by one mutation using one way ANOVA analysis. We then plotted those results on maps using arrows that connect each pair of adjacent alleles. For each comparison of adjacent alleles, we indicate the one whose expression resulted in the fastest growth by directing the arrowhead towards that variant, and implying that evolution would proceed in that direction if the two variants occurred simultaneously in a population. In other words, the one indicated by the arrowhead would increase in frequency and reach fixation in the population, while the other would be lost.

Red arrows indicate significance, and black arrows indicate differences that were not statistically significant by ANOVA, but that may still exist if a more sensitive assay was used.