Title:
Understanding the Effects of Sub-Inhibitory Antibiotic Concentrations on the Development of β-Lactamase Resistance Based on Quantile Regression Analysis

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Abstract:

Aims:

Quantile regression is an alternate type of regression analysis that has been shown to have numerous advantages over standard linear regression. Unlike linear regression, which uses the mean to fit a linear model, quantile regression uses a data set's quantiles (or percentiles), which leads to a more comprehensive analysis of the data. However, while relatively common in other scientific fields such as economic and environmental modeling, it is infrequently used to understand biological and microbiological systems.

Methods and Results:

We analyzed a set of bacterial growth rates using quantile regression analysis to better understand the effects of antibiotics on bacterial fitness. Using a bacterial model system containing 16 variant genotypes of the TEM β-lactamase enzyme, we compared our quantile regression analysis to a previously published study that uses the Tukey's range test, or Tukey Honestly Significantly Different (HSD) test. We find that trends in the distribution of bacterial growth rate data, as viewed through the lens of quantile regression, can distinguish between novel genotypes and ones that have been clinically isolated from patients. Quantile regression also identified certain combinations of genotypes and antibiotics that resulted in bacterial populations growing faster as the antibiotic concentration increased- the opposite of what is expected. These analyses can provide new insights into relationships between enzymatic efficacy and antibiotic concentration.

Conclusions:

Quantile regression analysis enhances our understanding of the impacts of sublethal antibiotic concentrations on enzymatic (TEM β-lactamase) efficacy and bacterial fitness. We illustrate that quantile regression analysis can link patterns in growth rates with clinically relevant mutations and provides an understanding of how increasing sub-lethal antibiotic concentrations, like those found

in our modern environment, can affect bacterial growth rates and provide insight into the genetic basis for varied resistance.

Significance:
Quantile regression analysis provides a distinctive way of analyzing bacterial growth rate data that has not yet been done and offers unique advantages to microbiologists. Identifying the mutations that are most likely to appear in the clinic can help scientists and epidemiologists better predict the directionality of antibiotic resistance and develop novel pharmaceuticals to combat this worldwide crisis.

Introduction

Antibiotic resistance is a serious public health threat and responsible for over thirty-five-thousand deaths worldwide in 2019 (Arnold, Thom et al. 2011, Frieri, Kumar et al. 2017, Centers for Disease Control and Prevention 2019, Iwu, Korsten et al. 2020, Kaur, Pham et al. 2021). Due to the abuse of antibiotics, multi-drug-resistant pathogens have been rising across the globe (Zhuang, Achmon et al. 2021). Bacteria are regularly exposed to sub-inhibitory concentrations of antibiotics found in wastewater from agriculture (Leiva, Pina et al. 2021, Sanz, Casado et al. 2021) and from clinics (Bojar, Sheridan et al. 2021). These sub-inhibitory concentrations of antibiotics accelerate the diversification of resistance genes (Li, Phulpoto et al. 2021, Li, Xia et al. 2021, Byun, Ha Han et al. 2022, Xu, Tan et al. 2022) and increase the prevalence of antibiotic-resistant pathogens more than lethal concentrations do (Mira, Meza et al. 2015, Mira, Østman et al. 2021, Byun, Ha Han et al. 2022).

B-lactam antibiotics are the most widely prescribed antibiotics worldwide because of their efficacy and low toxicity to human and animal organisms (Demain and Elander 1999). However, due to their abuse, β-lactam resistance is prominent. As a result, the most studied group of resistance genes are those that encode for β-lactamases, enzymes that cleave the active site of β-lactam antibiotics and render them inactive (Poole 2004). To combat increasing resistance, different types of β-lactam antibiotics have been developed that differ structurally but have the same mechanism of action, ranging from early penicillins to fourth generation cephalosporins and β-lactam with the β-lactamase inhibitors. Typically, the newer the antibiotic generation, the more complex the chemical structure and/or the more effective against bacteria that produce β-lactamases.

In an earlier study (Mira, Østman et al. 2021), we investigated the effects of sub-inhibitory concentrations of β-lactam antibiotics on two representatives from the penicillin group, representatives from all four generations of cephalosporins, and one penicillin with beta-

lactamase inhibitor on the selection of different combinations of the four point mutations within a TEM β-lactamase resistance gene, *bla*$_{\text{TEM-85}}$. Briefly, (Mira, Østman et al. 2021) measured the growth rate of 16 TEM genotypes that contained different combinations of the four substitutions found in *bla*$_{\text{TEM-85}}$ in the presence of three sub-inhibitory concentrations of β-lactam antibiotics. Using the Tukey honestly significant difference (HSD) test, we calculated all pairwise p-values between growth rates across antibiotic concentrations. Surprisingly, we were able to identify significant increases in growth rates as antibiotic concentration increased for 10 β-lactam antibiotics.

The Tukey HSD, like standard linear regression, relies on the assumption that variance is equal across the groups associated with each mean—also referred to as homogeneity of variance. However, errors are often non-normally distributed, which can happen when response variables behave differently at varying levels of the independent variable. For example, growth rates could differ markedly at low versus high concentrations of antibiotics due to the genetic background of the bacterial population, mutation rates, or limiting effects of the antibiotic. For this reason, we re-examine our data from this earlier study using quantile regression analysis.

Quantile regression analysis is an alternative approach to studying the relationships between response and independent variables first proposed by Koenker (Koenker 1978). As the name implies, quantile regression uses quantiles (also known as percentiles) within a dataset, rather than just the mean, and can provide a more comprehensive analysis (De Oliveira, Fischmeister et al. 2013, Chen, Bertke et al. 2021). In clinical studies, quantile regression can identify trends not otherwise seen in standard linear regression, such as differences between high- and low-risk patients (Staffa, Kohane et al. 2019). Quantile regression has been particularly useful in ecology (Thomson, Weiblen et al. 1996), where limiting factors (uncontrolled factors that are not measured but nonetheless influence the response variable) arise in dealing with ecological and/or biological datasets. In our case, limiting factors include the mutations, and different combinations of mutations, that are present in the TEM β-lactamase gene. In spite of its many advantages, only a few studies have applied quantile regression to biological datasets (Villain, Lozano et al. 2014). In addition, we are unaware of any study that uses quantile regression analysis on microbial growth rates over time or considers limiting factors on microbial growth rate, even though limiting factors are prevalent in microbial fitness datasets.

In this study, we analyze previously published bacterial growth rate data (Mira, Østman et al. 2021) to investigate whether quantile regression can enhance our insight into the effects of sub-inhibitory drug concentrations on the selection of point mutations within a TEM β-lactamase resistance gene, *bla*$_{\text{TEM-85}}$. Specifically, we use quantile regression analysis to investigate the effects of sub-inhibitory antibiotic concentrations on the evolution of TEM resistance genes, which other approaches that assume normally distributed errors may have missed. The differences across quantiles provide a more complete picture of the association between

antibiotic concentration and growth rates and how point mutations affect enzymatic efficacy, which may be linked to bacterial fitness. Overall, these analyses can lead to an improved understanding of the impact of sublethal concentrations of antibiotics in the environment on bacterial fitness.

Methods

Quantile Regression Analysis

To investigate whether quantile regression analysis provides more insight into bacterial growth rates than standard linear regression, we used a dataset containing growth rates of variant TEM genotypes on the pBR322 plasmid expressed in *E. coli* strain DH5αE (Table 1) (Goulart, Mahmudi et al. 2013). Specifically, we used all 16 combinations of the four amino acid substitutions present on the β-lactamase gene $bla_{\text{TEM-85}}$ (Mira, Østman et al. 2021), only 10 of which have been clinically identified (Jacoby 2020)(Table 1). These genotypes range from the wild-type TEM-1, that confers resistance to only penicillins, four individual single substitutions that each carry their own resistance phenotype based on the location of the substitution in the TEM enzyme, the six possible combinations of double mutations, four possible triple combinations and finally TEM-85 that contains all four substitutions and has evolved to confer resistance to penicillins as well as higher generations of cephalosporins (Mira, Østman et al. 2021) (Table 1). It is important to note that these genotypes all carry individualized efficacy to different generations of β-lactam antibiotics depending on the location of the point mutation within the TEM enzyme and any interactions the amino acid residues have with one another. We exposed each of the 16 variants to various sublethal concentrations of 12 β-lactam antibiotics for 22 hours (Table 2). The β-lactam antibiotics represented all generations, from early penicillins to fourth generation cephalosporins and β-lactam + inhibitor combinations. The growth rates were calculated using the *growthrates* package (Hall, Acar et al. 2014).

Creating quantile regression plots

We first normalized and plotted the growth rate data to the highest concentration of each antibiotic, scaling the growth rate values by a factor of 1000 to make the analysis easier and to control for numerical ill-conditioning (top panel, Figure 1). We then calculated the slopes of the lines of best fit through each quantile (20^{th}, 40^{th}, 60^{th}, and 80^{th}). Using the *quantreg* package in R, we then generated quantile regression plots that represent these slopes at each quantile (bottom panel, Figure 1).

Identifying genotypes with non-normal distributions of growth rate

Using the quantile regression plots for each antibiotic, we identified genotypes that had differences in distribution—i.e., those in which quantile regression analysis would be most useful. For the purposes of this study, we focus on non-normally distributed data to better

understand effects across quantiles. We visualized quantile regression plots using the *rq* function from Koenker's *quantreg* package in R and identified four types of trends, representing how the distribution of the data changes across quantiles (Figure 2). A constant trend (Figure 2, Panel A) is normally distributed data and represents genotypes that grew at the same rate- indicating stable enzymatic activity- across the concentrations of antibiotic. An increasing trend (Figure 2, Panel B) indicates non-normally distributed data at the higher quantiles and represents genotypes that led to an increase in enzymatic activity the faster the bacteria grew. Contrarily, a decreasing trend (Figure 2, Panel C) indicates non-normally distributed data at the higher quantiles and represents genotypes that led to a decrease in enzymatic activity the faster the bacteria grew Finally, a U-shaped trend (Figure 2, Panel D) indicates non-normally distributed data at both the higher and/or lower quantiles with a dip in the middle quantiles. This type of trend represents genotypes that led to fluctuations of enzymatic activity as the antibiotic concentrations increased. It is important to note the sign of the slopes (y-axis). In some cases, for any trend, the slopes at quantiles are positive, indicating that as the antibiotic concentration increased, so did the growth rates. These distribution patterns in the quantile regression plots can give us insight into the efficacy of the TEM enzyme in the presence of increasing concentrations of certain antibiotics.

Results

In order to test the hypotheses that analyzing different quantiles of bacterial growth might provide a more complete picture of the relationship between antibiotic concentrations and bacterial fitness, we examined distribution patterns in the quantile regression plots across genotypes (Figure 3). Across all genotypes (gray bars, Figure 3), we found ~32% constant trends, meaning that growth rate data was normally distributed across increasing antibiotic concentrations in 32% of antibiotic treatments. Genotypes that have been clinically identified account for 71% of the normally distributed data. However, most of the data (~68%) showed non-normal distributions (i.e., increasing, decreasing, or U-shape trends) shown by the green, red, and yellow bars, respectively (Figure 3).

To investigate whether certain antibiotics or β-lactam generations produce more non-normal distributions in growth rate data, we visualized the trend for each genotype-antibiotic pair (Figure 4). We found that ampicillin (AMP) and ampicillin + sulbactam and piperacillin + tazobactam (SAM, TZP) had largely decreasing trends. AMP, SAM, and TZP had more of an impact on growth rates in higher quantiles than lower quantiles, which means that the faster the bacteria grew, the more the antibiotic treatment inhibited their growth.

Second-generation cephalosporins cefaclor, cefotetan and cefuroxime (CEC, CTT, and CXM) resulted in mostly increasing trends. These antibiotics had less of an impact on growth rates at higher quantiles compared to lower quantiles. In other words, the slower the bacteria grew, the more the antibiotic inhibited their growth. Cefaclor (CEC) showed mostly increasing trends across all genotypes, specifically in genotypes that encode L21F and/or R164S substitutions

(L21F, R164S, LR, RE, RT, LRT, RET, and TEM-85). Cefotetan (CTT) provided similar results, with largely increasing trends for amino acid substitutions L21F, E240K, and T265M (LE, LT, RT, LRE, LET, TEM-85).

Neither third- nor fourth-generation cephalosporins showed a prevalent pattern in their quantile regression trends. We noticed a similar number of increasing and decreasing trends, with slightly more decreasing trends overall. However, the genotype that carries the single amino acid substitution R164S exhibited increasing trends across 80% of third generation cephalosporins. We also noticed a shift in trends in the fourth-generation cephalosporin cefepime (FEP) as the number of substitutions increased (Figure 4). The pattern changed from consistent decreasing trends in TEM-1 wildtype, single-substitution, and double-substitution genotype variations to increasing trends in two triple-substitution genotypes and the four-substitution TEM-85. This indicates that the β-lactamase enzyme became more efficient at hydrolyzing FEP at higher concentrations as the number of amino acid substitutions increased.

Next, we compared the clinically isolated genotypes to those that have not yet been clinically isolated to see if there was any significant difference in quantile regression patterns that correlated to the TEM enzyme's ability to hydrolyze β-lactam antibiotics. We found that the genotypes that have not been clinically isolated had more decreasing trends across all antibiotics (46%) than clinically identified genotypes (26%; compare red boxes for genotypes without and with an asterisk in Figure 4). Decreasing trends indicate that the faster the bacterial populations grew, the more inefficient the TEM enzyme was at hydrolyzing the antibiotics or the more effective the antibiotics were at inhibiting growth. However, accumulated mutations in populations with decreasing trends may have resulted in faster growth unrelated to the TEM enzyme's ability to hydrolyze antibiotics. Clinically and non-clinically isolated genotypes had similar frequencies of increasing trends (22% and 26%). In these cases, when the bacterial populations grew faster, the TEM enzyme was able to sufficiently hydrolyze increasing antibiotic concentrations.

Finally, we identified patterns in genotypes or antibiotics that resulted in a positive slope across some or all quantiles. A positive slope indicates that growth rates increased along with antibiotic concentration - the opposite of what is expected when exposing bacterial populations to antibiotics. Apart from cefotetan and ceftazidime (CTT, CAZ), all other antibiotics had at least one genotype that exhibited a positive slope (Figure 4). Approximately 27% of all treatments (13 antibiotic treatments X 16 genotypes) had positive slopes at some or all quantiles. Half of these positive slopes occurred across all quantiles, 29% at higher quantiles (50^{th} to 90^{th}) and 21% at lower quantiles (10^{th} to 40^{th}) (Figure 4). There was no direct correlation between the quantile regression trends and positive slopes. However, nearly all genotypes (12/16) had a positive slope in part or all the quantiles in the presence of the only penicillin we tested, ampicillin (AMP). We

also noticed genotypes that have been clinically identified had positive slopes across all quantiles more often than non-clinically identified genotypes.

Discussion

In contrast to our earlier study (Mira, Ostman et al. 2021), which assumed homogeneity of variance and used the Tukey HSD test, our quantile regression analysis of the same dataset has provided more robust findings related to amino acid substitutions and enzyme efficacy, antibiotic class, and whether genotypes have been clinically identified.

Typically, as antibiotic concentration increases, bacterial growth rate decreases. However, some bacteria can hydrolyze antibiotics so quickly that they can use the degraded antibiotics as a carbon source for continued growth and proliferation (Dantas, Sommer et al. 2008). This is the case for some TEM genotypes (Mira, Meza et al. 2015, Mira, Østman et al. 2021). Authors previously showed that growth rate increased as antibiotic concentration increased in response to at least one antibiotic for all but two TEM genotypes: T265M and LET. However, our quantile regression analysis revealed that all genotypes had positive slopes across some or all quantiles, including T265M and LET- meaning the presence of these amino acid substitutions allowed the bacterial populations to grow faster as antibiotic concentration increased. LET only had positive slopes at the lower quantiles for the penicillin/inhibitor treatment piperacillin + tazobactam (TZP), which suggests that the slower the bacteria grew, the better the TEM enzyme with the L21F/ E240K/T265M substitutions was at hydrolyzing TZP and using the degraded antibiotic product as a carbon source for faster growth.

The location of amino acid substitutions within the TEM enzyme can influence the enzyme's efficacy, even if not located in the binding site. For example, the genotype with the single substitution T265M had positive slopes across all quantiles in the presence of ampicillin (AMP), in upper quantiles in the presence of cefuroxime (CXM), and in lower quantiles in the presence of cefprozil (CPR). CXM and CPR are second- and third generation cephalosporins, so their structures are more complex than AMP, a penicillin class antibiotic. The T265M substitution is distant from the binding site of the enzyme, positioned on the outer face of the β-sheet but buried by surrounding amino acids (Knox 1995). Recently, this single mutation has been identified clinically and has shown resistance to β-lactam inhibitor treatments like piperacillin + tazobactam (TZP) and resistance to some third-generation cephalosporins (Mulvey and Boyd 2009). This clinical isolate also had a G162T mutation in promoter region *P4*, resulting in hyperproduction of the TEM gene; this could explain the resistance to β-lactamase inhibitor treatments (Mulvey and Boyd 2009). In this study we used *E. coli* strain K12, which lacks the *P4* region, potentially explaining why we did not see positive slopes for T265M in any β-lactam inhibitor treatments.

Our analysis reveals that TEM genotypes that have been identified in the clinic have more positive slopes across all antibiotic treatments than non-clinically identified genotypes, particularly in the presence of AMP. Genotypes are typically clinically identified when they are highly selected for in natural environments (Shawky, Suleiman et al. 2021), which can correlate with a more stable and effective enzyme. In fact, we find that clinical isolates had normal distributions across all quantiles in 71% of treatments, compared to only 32% among non-clinically isolated genotypes. This suggests that normal distributions across all quantiles correlates with more-stable enzymatic activity throughout populations.

R164S is the most common amino acid substitution observed within the more than 220 clinically identified TEM β-lactamases and is highly selected for because it increases enzyme efficacy in response to higher-generation cephalosporins. The 164th amino acid position is just below the binding site that contains the catalytic glutamate at position 166 (Herzberg 1991, Knox and Moews 1991). In R164S, serine (S) replaces arginine (R), resulting in a hydrogen-bonding amino acid with one less hydrogen bond donor (Palzkill, Le et al. 1994). This reduction in the number of hydrogen bonds eradicates electrostatic attraction and allows for more flexibility in the binding site, creating more space for the bulky sidechains of third- and fourth-generation cephalosporins (Escobar, Tan et al. 1994). We found increasing trends in R164S populations for five cephalosporin treatments—the highest number of increasing trends across all 16 genotypes tested. Increasing trends mean that the faster a bacterial population grows, the better it is at hydrolyzing the applied antibiotic. R164S showed no increasing trends in penicillin or penicillin + inhibitor treatments, supporting the idea that R164S is selected for cephalosporin resistance.

In comparison to clinical isolates, non-clinically identified genotypes are suspected to have less stable or effective enzymes. This likely has to do with the amino acid substitution locations and their impact on the three-dimensional structure of the enzyme and is reflected in the distribution of growth rate data and quantile regression trends. For example, genotypes LE, LRE, and LET have the double substitution LE (L21F and E240K) in common and have not been clinically identified. The first amino acid substitution, L21F, is a recently identified point mutation in TEM-117, a TEM β-lactamase first clinically isolated in 2003; no current research demonstrates L21F's role in TEM β-lactamase (Box, Paauw et al. 2002, Zeil, Widmann et al. 2016). Although no work has been done on L21F as a single mutation, it has been clinically identified with other amino acid substitutions within TEM β-lactamase, though not solely with E240K (Goussard and Courvalin 1999, Baraniak, Fiett et al. 2005, Morris, Whelan et al. 2006, Drissi, Ahmed et al. 2008). E240K is positioned at the end of β-strand B3 such that the hydrophilic glutamic acid (E) residue is exposed, allowing it to interact with the acylamide substituents of cephalosporins (Knox 1995). However, B3 is too far in proximity to interact with smaller ligands like the inhibitors (clavulanic acid or sulbactam). Therefore, substitutions at this position typically do not appear in inhibitor-resistant TEM variants (Knox 1995). Overall, the distribution of growth rate data and trends in the quantile regression analysis reflect likely enzyme instability in LE genotypes. The genotypes that include LE have the highest numbers of decreasing trends in their

quantile regression plots (LE has eight, and LRE and LET both have seven) across all antibiotics. This means that the higher the antibiotic concentration, the slower these genotypes grew, suggesting an enzyme less effective at hydrolyzing β-lactam antibiotics. Likewise, genotypes with LE combination also had positive slopes at lower quantiles in the presence of penicillin + inhibitor (TZP) and a second-generation cephalosporin (CEC). This indicates the slower the populations grew, the TEM β-lactamase enzyme was more effective at hydrolyzing TZP and CEC. Altogether, these results suggests that the combination of L21F and E104K are selected for at lower concentrations of antibiotics, highlighting the influence of sub-lethal concentrations of antibiotic in the environment on the evolution of antibiotic resistance.

Conclusion

While infrequently used for biological datasets and not previously used for bacterial growth rate data, our study demonstrates that quantile regression analysis offers unique advantages to microbiologists. Our results show that quantile regression analysis provides a more detailed understanding of how increasing sub-lethal antibiotic concentrations, similar to those found in the environment, affect bacterial growth rates and provides insight into the genetic basis for varied responses. We illustrate how quantile regression analysis can link patterns in growth rates with certain types of β-lactam antibiotics and with clinically relevant mutations that can either hinder or enhance enzymatic activity on antibiotics. Ultimately, identifying the mutations that are most likely to appear in the clinic can help scientists and epidemiologists better predict the directionality of antibiotic resistance and develop novel pharmaceuticals to combat this worldwide crisis.

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Authors' contributions
Portia Mira analyzed the data and lead writing.
Candace C. Cole collected additional data that was needed for the analysis and revised the manuscript.
Juan C. Meza suggested the use of quantile regression, implemented the R code, analyzed the data, and contributed to writing the manuscript.

Availability of data
All data and code used in this work can be found on GitHub upon acceptance.

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There are no conflicts of interest.

Ethics approval
Not applicable

Consent to participate
All authors consent to the participation of this work.

Consent for publication
All authors consent to the publication of the work.

Figure Legends

Figure 1: Representative data showing how quantile regression plots are made.
Top: Growth rate (cells/minute X 10^{-3}) plotted against normalized antibiotic concentration. X-axis ranges from 0 (no antibiotic) to 1 (highest concentration of antibiotic). Four quantiles are plotted (80^{th} quantile – red, 60^{th} quantile – orange, 40^{th} quantile – blue, 20^{th} quantile - black) across the growth rate data. **Bottom:** Quantile regression plots show the slope of each quantile line plotted across all quantiles. The colored dots represent the slope of the best fit model of each line in the above graph which corresponds to each of the quantiles. The red solid horizontal line represents data that would be normally distributed with the 95% confidence interval of normally distributed data shown by the red dotted lines. The gray shaded area represents the variation of the data calculated using the quantile regression package.

Figure 2: Four common trends of quantile regression plots. A) Constant: a trend remaining within the confidence intervals (red dotted lines) of the quantile regression plot. This type of trend means that the slope of the line of best fit at each quantile remains within the confidence intervals of a standard linear regression. Constant trends signify that the data is normally distributed and that a standard linear regression would work well. Variation that deviates beyond the red dotted lines signifies that the data is not normally distributed and that there are different effects at different quantiles. This example is given with the genotype R164S/E240K in the presence of cefepime (FEP) **B) Increasing:** a trend from bottom left to upper right. In this trend type, lower quantiles have more-negative slopes than higher quantiles. In our study, this trend means that higher antibiotic concentrations have a more detrimental effect on bacterial populations that grow slower (lower quantiles) compared to populations that grow faster (higher quantiles). This example is given with the genotype L21F/R164S in the presence of cefprozil (CPR) **C) Decreasing:** a trend from upper left to bottom right; the opposite of the increasing trend described above. Here, lower quantiles have less-negative slopes than higher quantiles, meaning that higher antibiotic concentrations have a more detrimental effect on bacterial populations that grow faster (higher quantiles) than on those that grow slower (lower quantiles). This example is given with the genotype L21F in the presence of cefotaxime (CTX) **D) U-shaped:** the extreme ends of this trend (80^{th} and 20^{th} quantiles) have less-negative slopes and the middle quantiles (25^{th} to 75^{th} quantiles) have more-negative slopes. This means that higher antibiotic concentrations are less detrimental to the bacterial populations that grow fastest and slowest compared to those in the middle. This example is given with the genotype R164S/E240K/T265M in the presence of ceftriaxone.

Figure 3: Frequency of quantile regression trend types for TEM-85 genotypes across all antibiotic treatments. The number of occurrences of each trend type across the 16 TEM genotypes. Total number of U-shaped trends are yellow, constant trends are gray, decreasing

trends are red, and increasing trends are green. The genotypes that have been clinically identified are marked with an asterisk (*).

Figure 4: Quantile regression trends and positive slopes for TEM-85 genotypes across antibiotic treatments. Antibiotics are listed by increasing chemical complexity and class (first column) and identified by abbreviation (second column). Genotypes that have been clinically identified are marked with an asterisk (*). Genotypes are listed in subsequent columns in order of increasing number of substitutions, from wild-type (TEM-1) to TEM-85, which contains all four substitutions. The count of each trend type by antibiotic is listed in the table to the right.

Tables

Table 1: Variant TEM genotypes.

Genotypes are listed by increasing number of substitutions, from TEM-1 (no substitutions, wildtype) to TEM-85 (four amino acid substitutions). The number of substitutions is listed in the first column; full amino acid substitutions are listed in the second column. If the genotypes have been clinically isolated, their TEM name (third column) and year of first isolation (last column) are denoted

No. of Substitutions	Substitution	Isolated	Year
0	TEM-1	TEM-1	1965
1	L21F	TEM-117	2003
1	R164S	TEM-12	1999
1	E240K	TEM-191	2011
1	T265M	TEM-168	2009
2	L21F/R164S	TEM-53	1999
2	L21F/E240K	-	-
2	L21F/T265M	TEM-110	2002
2	R164S/E240K	TEM-10	1989
2	R164S/T265M	-	-
2	E240K/T265M	-	-
3	L21F/R164S/E240K	-	-
3	L21F/R165S/T265M	TEM-102	2003
3	L21F/E240K/T265M	-	-
3	R164S/E240K/T265M	-	-
4	L21F/R164S/E240K/T265M	TEM-85	2005

Table 2: Antibiotics and concentrations used to measure bacterial growth rates

Antibiotics are listed in order of increasing complexity (β-lactam generation) in the first column, followed by full antibiotic name and abbreviation. The three concentrations used in (Mira, Østman et al. 2021) are listed in the last column.

Antibiotic Name	Abbreviation	Concentrations (μg/mL)	β-lactam group
Amoxicillin	AMX	256, 512	Penicillin
Ampicillin	AMP	1024, 2048, 3072	Penicillin
Ampicillin + Sulbactam	SAM	8, 16, 32	Penicillin + β-lactamase Inhibitor
Piperacillin + Tazobactam	TZP	32, 64, 128	Penicillin + β-lactamase Inhibitor
Cefaclor	CEC	2, 4, 8	2nd generation cephalosporin
Cefotetan	CTT	0.063, 0.125, 0.25	2nd generation cephalosporin
Cefuroxime	CXM	2.25, 3, 4	2nd generation cephalosporin
Ceftazidime	CAZ	0.125, 0.25, 0.5	3rd generation cephalosporin
Cefprozil	CPR	8, 12, 16	3rd generation cephalosporin
Ceftriaxone	CRO	0.025, 0.05, 0.1	3rd generation cephalosporin
Cefotaxime	CTX	0.03, 0.06, 0.123	3rd generation cephalosporin
Ceftizoxime	ZOX	0.0078, 0.0156, 0.03	3rd generation cephalosporin
Cefepime	FEP	0.0312, 0.0625, 0.125	4th generation cephalosporin