Are antibiotic breakpoints globally consistent, does it matter if not? (no, yes)

Abstract: **a) Objectives**: Antibiotic resistance is quantified in practice by 'breakpoints', our objective is to assess whether these are defined rationally, or not, using ATLAS data. Breakpoints are pairs of drug dosages by which clinical microbiologists mark the dosing limits blow which treatments are expected to fail and above which they are likely to succeed. Three organisations in the US (CLSI, FDA, USCAST) and one in Europe (EUCAST) publish breakpoints and their expert committees have decided, tacitly, that different continents should have different breakpoints. Thus resistance, and recommended dosing, differ across the planet and when regions have changed their adherence to one of these organisations, this has lead to abrupt changes in local dosing recommendations. Unfortunately, how this framework affects treatment outcomes remains largely unexplored due to a lack of treatment outcome data. **b) Methods**: First, we determine the correlation between different continental breakpoints, then we ask whether those correlations are reasonable given the minimal inhibitory concentration (MIC) data in ATLAS. **c) Results**: Using Bayes PCA to impute missing ATLAS data for Enterobacterales species, we find supervised learning classifiers can distinguish pathogen-antibiotic (PA) pairs based on their MIC data, despite having identical breakpoints. We then identify PA pairs with similar MIC distributions but different breakpoints, and vice versa. Using artificial neural networks (ANNs) to model breakpoint decisions based on MIC data, we apply optimisation algorithms to ANNs to predict which data structures have the greatest uncertainty when setting breakpoints, finding that a 'selective sweep' from the theory of population genetics is a candidate for this. **d) Impact of the work**: We show the governance principles for global breakpoint-setting are neither self-consistent within organisations, nor mutually consistent between organisations based on ATLAS data. We would therefore recommend that breakpoint-setting organisations revisit and refine the algorithmic methodologies that support breakpoints. Our analysis is limited by a lack of treatment outcome data and we caution that because of their clustered structures, MICs alone are not optimal for differentiating between successful and unsuccessful treatments. **e)** 8 figures and 1 table are placed at an appropriate position in the text.

S/R breakpoints	*K.pneumoniae*	*E.coli*	*E. cloacae*	*K.pneumoniae*	*E.coli*	*E. cloacae*
organisation	CLSI	CLSI	CLSI	EUCAST	EUCAST	EUCAST
Amikacin	4/5.05	4/5.05	4/5.05	3/3	3/3	3/3
Amoxycillin clavulanate	3/4.05	3/4.05	3/4.05	7.67/7.67	7.67/7.67	7.67/7.67
Ampicillin	3/5	3/4.05	3/5	3/3	3/3	3/3
Azithromycin	-/-	**4/8.05**	**-2/-2**	-/-	-/-	-/-
Aztreonam	2/4	2/4	2/4	0/2	0/2	0/2
Cefepime	1/4	1/4	1/4	0/2	0/2	0/2
Ceftaroline	-1/1	-1/1	-1/1	-1/-1	-1/-1	-1/-1
Ceftazidime	2/4	2/4	2/4	0/2	0/2	0/2
Ceftazidime avibactam	3/4	3/4	3/4	6.41/6.41	6.41/6.41	6.41/6.41
Ceftriaxone	0/2	0/2	0/2	0/0.58	0/0.58	0/0.58
Doripenem	0/2	0/2	0/2	0/1	0/1	0/1
Ertapenem	-1/0.05	-1/0.05	-1/0.05	-1/-1	-1/-1	-1/-1
Imipenem	0/2	0/2	0/2	0/2	0/2	0/2
Levofloxacin	1/2.05	1/2.05	1/2.05	-1/0	-1/0	-1/0
Meropenem	0/2	0/2	0/2	1/2.32	1/2.32	1/2.32
Minocycline	2/4	2/4	2/4	-/-	-/-	-/-
Piperacillin tazobactam	4/7	4/7	4/7	6.39/6.39	6.39/6.39	6.39/6.39
Tigecycline	1/3	1/3	1/3	-/-	-/-	-/-

Fig 1 S & R breakpoints for the stated species for each organisation (units of $\log_2 \mu g/mL$). Most breakpoints are the same between species but differ between organisations, aside from Azithromycin and Imipenem. Azithromycin differs between species and between organisations, Imipenem is the same in all cases.

Introduction: visualising breakpoints across historical MIC data. Within-country laboratory data support the assertion that microbial pathogens are increasingly resistant to antibiotics [1]. Controlled antibiotic susceptibility tests (ASTs) define what resistance means here: the output of ASTs, namely the minimal inhibitory concentration (MIC), is the lowest dose at which pathogen growth cannot be detected over a defined period of time and 'resistance' arises when the MIC is observed above a given published CLSI or EUCAST breakpoint. Laboratory MICs provide an imperfect proxy for clinical resistance, and, therefore, treatment outcome, but historical MIC values are curated by CLSI and EUCAST to help its expert members decide where breakpoints delineate between drug susceptible (S) and resistant pathogens (R). When a patient isolate is classified as S or R according to whether its MIC lies above a breakpoint, an informed treatment can then proceed. Additional classifications use 'intermediate-like' states, I, whose definitions vary markedly between organisations, but we omit these states from the majority of our discussion below.

Determining breakpoints is not straightforward because of the factors involved in taking *in vitro* measurements into a patient context. Tissue penetration of the molecule [2] is vital to achieve effective dosing so breakpoints can differ between infection sites, even for the same PA pair. Importantly, human genetics play a role in maintaining effective doses, for instance exogenous metabolism mediated by cytochrome p450s is polymorphic among humans [3], and this can affect the *in vivo* bioavailability and PKPD of antibiotics within patients from different countries. This could, in theory, support different continental breakpoints.

Indeed, prior, and much smaller studies than ours have shown CLSI breakpoints, as defined in the US, and EUCAST breakpoints, as defined in Europe, differ. The authors of [4] state '*[continental] harmonization is urgently needed*' but this comment is not based on whether the breakpoint *status quo* is expected given the available MIC data, so we will test this using ATLAS. Unfortunately, it cannot be tested directly using MIC datasets curated by CLSI and EUCAST due to an absence of metadata. Herein we explain why we find no evidence in ATLAS to support the current use of different breakpoints on different continents.

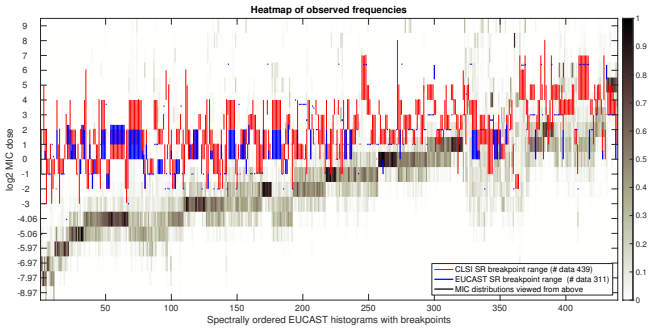

Fig 2 Visualising breakpoints: EUCAST MIC distributions (i.e. 1d histograms) of 439 PA pairs are 'stacked' along the x-axis to create a 2d black-grey-white heatmap where darker regions have higher frequencies of pathogens with the MIC values on the y-axis. More highly resistant PA pairs appear top-right and more drug-susceptible ones appear bottom left. Note how black regions increase from low to high MIC values but breakpoints (red and blue vertical lines denote the S-to-R breakpoint range) do not. This indicates there is a moderate correlation between the two because MIC data vary more than breakpoints do, indeed the latter tend to reside around -2 to $4\log_2 \mu g/mL$ whereas MICs can go much lower (where black is below red/blue).

Results 1: weak EUCAST / CLSI correlations in the context of ATLAS. MIC data alone are insufficient for our study, so we use tables of breakpoints from CLSI and EUCAST. USCAST also publishes a list (here) defined by either species or genera. One observation about breakpoints is critical: Enterobacterales species have the same EUCAST breakpoints and very similar CLSI ones, but their values can differ between organisation (e.g. Fig 1).

To compare cross-continental breakpoints at the species level, we accessed historical MIC distributions curated by EUCAST and, to create a breakpoint list for analysis, we used fuzzy text-matching to determine corresponding PA pairs between CLSI and EUCAST's databases. This resulted in 558 CLSI S and R breakpoints, 439 of which have corresponding EUCAST MIC distributions. This also resulted in 381 EUCAST breakpoints, leaving 311 PA pairs with EUCAST breakpoints, historical MIC data and CLSI breakpoints. These values above differ both because we took a conservative approach to text-matching that avoided false positives and also because CLSI historical MIC distributions do not have the same form as EUCASTs', so we do not use them. We excluded 18 EUCAST S breakpoints of $-9.97\log_2 \mu g/mL$ as they are at the lower limit of the dosage range and so could introduce artefacts into our analyses.

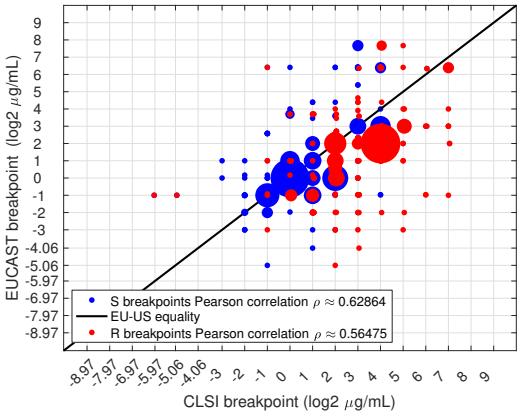

Fig 3 US-Europe breakpoint correlation coefficients: CLSI breakpoints (US-based, x-axis) and EUCAST breakpoints (Europe-based, y-axis), coefficients are stated in the legend.

Spectrally ordering EUCAST MIC distributions and superimposing their breakpoints yields Fig 2 from where we hypothesise that MICs and breakpoints correlate weakly. To see this, note in Fig 2 how CLSI (red) and EUCAST (blue) breakpoints reside in the range -1 to $4\mu g/mL$ and yet the black regions which indicate where modes of the MIC distributions for each PA pair are, tend to be much lower.

From data in Fig 2 we find correlation coefficients between CLSI and EUCAST S and R breakpoints which are $\rho \approx 0.56/0.63$ respectively, as observed in Fig 3. Interestingly, Fig 3 indicates some PA pairs' breakpoints differ by as much as $8\log_2$ units, supporting the hypothesis that US and EU-based patients might receive very different dosages for the same infection.

EUCAST and CLSI laboratory testing standards are different and ATLAS MICs satisfy the CLSI standard. This is important for our analysis and it leads to the question: is the weak continental breakpoint correlation (Fig 3) a downstream consequence of different MIC patterns in Europe and the US arising from the use of different lab standards? It is not possible to be definitive but we believe the answer may be no, as we argue from one perspective using Fig 4 which delineates Fig 3 according to antibiotic class. Fig 4 shows whether breakpoints for the PA pairs in each class bias towards CLSI or EUCAST: note how more antibiotics bias towards CLSI than to EUCAST because the former tend to be larger (Figs 3,4).

We reasoned one source of this bias could be the inoculum effect (IE) for β-lactam classes because CLSI and EUCAST publish different guidelines on permissible inoculum sizes. A careful *in vitro* study [5] shows the acceptable inoculum range to CLSI of 2×10^5 to 8×10^5 cells/ml can explain a $3\log_2$ variation in MIC. However Fig 4 shows that biases of this size can occur towards both CLSI and EUCAST for β-lactams, so the IE does not explain the US breakpoint bias in Figs 3 and 4. Nevertheless, Fig 4 lays the foundation for bringing ATLAS into our analyses because it contains the continental metadata labels that we need to design metrics that assess whether the above biases are consistent with real-world MIC data.

One basis for global breakpoint and MIC differences could be that strains of the same species circulating in the US exhibit a higher frequency of DNA or plasmid-based resistance mechanisms, perhaps due to higher antibiotic consumption, but testing this hypothesis would require genome sequencing data following access to ATLAS strains, which is feasible but beyond our scope.

Results 2: EUCASTs' Enterobacterales breakpoints are identical, CLSIs' are not: does ATLAS concur? Seeking justification for the different continental breakpoints, we compared continental MICs using ATLAS. For this we extracted all European and US-based patient MICs from ATLAS for all antibiotics for the 3 species *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*. Importantly, these Enterobacterales species have identical EUCAST breakpoints and almost-identical CLSI ones (Fig 1; and yet CLSI and EUCAST's differ). We then used Bayes PCA to compress their data dimension to 3, sufficient to account for over 99% of the variance (Fig 5). Bayes PCA imputes missing MICs under certain statistical assumptions, estimating MICs where susceptibility tests have not been performed. We have investigated the wider validity of this approach to predicting MIC values algorithmically but do not have the space to present that analysis here. However, as we applied the same procedures to both continents' MIC data, we now have 3-dimensional PCA datasets with 3 strain and 2 continent labels that we can investigate further.

Using Fig 5(left) we ask this supervised learning question: given PCA coefficients determined from an MIC data vector of unknown species and continent, with what probability can we predict the species and continent of origin for that pathogen? The bootstrapped 6x6 confusion matrix (Fig 5(right)) is diagonally dominant and addresses this for a K-nearest neighbour (KNN) classifier, the optimal one we were able to obtain for our data. It says this: the KNN classifier has an approximately 50% probability of identifying the correct species on the correct continent where a random null model has $1/6 \approx 16.66\%$ probability, but the KNN places the correct species on the incorrect continent with around 25% probability. The wrong species, irrespective of continent, is predicted in *circa* 25% of cases.

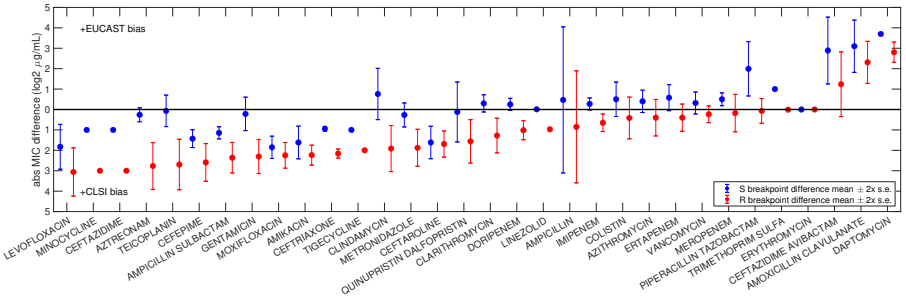

Fig 4 Several breakpoints bias towards the US, and 3 bias towards Europe: biases are shown by antibiotic class, mean and standard error (2x s.e.) are shown for PA pairs corresponding to each class.

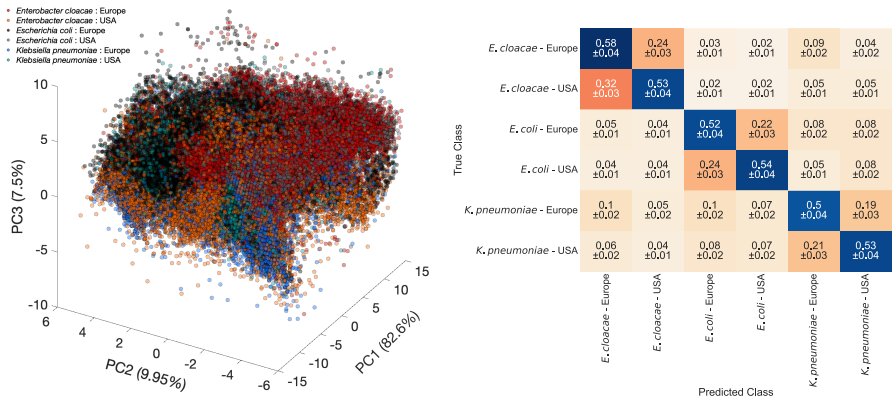

Fig 5 Using a KNN to predict species and continent from Bayes PCA coefficients of ATLAS MICs: (left) the first 3 PCA coefficients, (right) bootstrapped confusion matrix for the optimal classifier (a KNN). Approximately: where confusion does arise (1/2 of cases), the correct species is predicted to be in the wrong continent (1/4 cases), both continent and species are confused in 1/4 cases.

Thus, ATLAS data are sufficiently geometrically distinct in PCA space that we can recognise species from MICs alone with reasonably high probability and yet their breakpoints are the same within Europe and nearly so in the US (Fig 1). We therefore postulate that these 3 species could be treated differently when setting breakpoints, even though they currently are treated in unison. Remarks analogous to this have been made elsewhere but without supporting data analysis [4].

Results 3: ECOFFs as motivation for Gaussian Mixtures. At this point we can tentatively claim to have answered the question in our title: breakpoints are not globally consistent in a manner that our Machine Learning analysis of ATLAS predicts they should be and this matters for patients because breakpoints determine treatments. That said, we now present a different perspective to show that breakpoints currently in use are not entirely without logical foundation and, for this, we turn to Gaussian mixture models (GMMs).

Breakpoint setting begins with the 'ECOFF' (an 'ecological cutoff', documented here) which is an MIC value found by an algorithm [6] which, to approximation, seeks the upper tail of the most drug-susceptible cluster in the MIC distribution of a given PA pair. So, for lognormally distributed MIC data, our analysis (data not shown) indicates the ECOFF correlates strongly ($\rho \approx 0.973$) with the mean MIC + 2 standard deviations. However, the ECOFF algorithm is not reliable for multi-bump MIC distributions. So, motivated by the purpose of the ECOFF, we applied GMM to all historical EUCAST MIC data, finding that 3 clusters

was sufficient to describe the vast majority of the 3,206 datasets (based on AIC fitting criteria). We then hypothesised, again mirroring the ECOFF rationale, that GMMs would be structured in such a way that a low-MIC lognormal cluster (an 'S-cluster') would lie below the S breakpoint and a high-MIC lognormal cluster ('R-cluster') would lie above the R breakpoint. Fig 6 shows visually that this expectation is largely met, but a discussion of the exceptions to that expectation, which do exist, is beyond our scope.

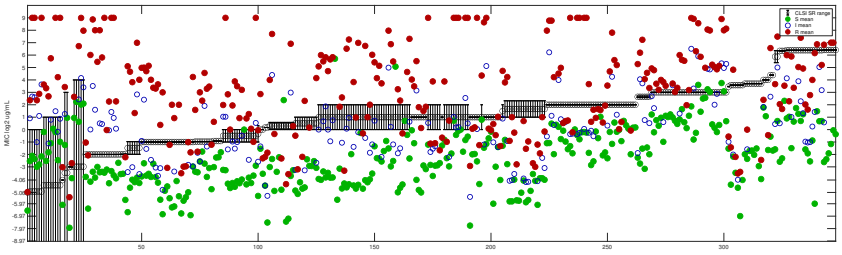

Fig 6 GMM R cluster centres typically lie above EUCAST breakpoints, S clusters lie below them: to show this AIC-optimal GMM models were fitted to all EUCAST MIC distributions with published breakpoints. Green dots are cluster centres for putative drug-susceptible 'S' clusters, red dots indicative putative resistance 'R' clusters and blue circles are intermediate. Vertical black lines denote EUCAST breakpoint ranges, from S to R values. Note how green dots tend to lie below the breakpoints and red dots tend to lie above them. Each x-axis value represents a different PA pair, y-axis values are centres of the GMM clusters.

Thus, there is a sense in which S and R breakpoints act as an imperfect classifier of GMMs into S and R clusters but we do not quantify that relationship here. We expected that linear modelling could be applied to GMM cluster centres to predict breakpoints but that correlation was moderate, $\rho \approx 0.64$, so we did not pursue this further.

Results 3: similar ATLAS MICs in US and Europe do not support breakpoint differences. We reasoned that breakpoint consistency could be quantified in two complementary ways: where similar MIC distributions have different breakpoints we call this a type I inconsistency and where different MIC distributions have similar breakpoints, this is a type II inconsistency. Writing $b(\mathbf{h})$ for some functional that defines breakpoints of a given MIC histogram, $\mathbf{h}$, and given a weighted Euclidean norm, $\|\mathbf{h}\|_w = \|w \cdot \mathbf{h}\|_2$, we quantify inconsistencies using the Lipschitz coefficient of b, $L_b(\mathbf{h}, \mathbf{k}) := |b(\mathbf{h}) - b(\mathbf{k})|/\|\mathbf{h} - \mathbf{k}\|_w$. L_b quantifies the proximity of breakpoints for a given proximity of MIC data, given w, and we expect, ideally, these two proximities should correlate strongly, so do they? (Aside: how might an ideal / fair 'breakpoint function', $b(\mathbf{h})$, be designed?)

As one is free to choose the weight, w, we did the following. Consider the ensemble of MIC data for all PA pairs, $\boldsymbol{H}$, which is an $M \times N$ matrix, and the M-dimensional vector of breakpoints $\boldsymbol{b}$. Here $N = 19$ is the number of MIC dosages and $M \approx 500$ is the number of breakpoints. Now solve (as an ℓ^2-optimality problem) the positivity-constrained linear regression $\boldsymbol{b} \approx \alpha \cdot \mathbf{1} + \beta \cdot \boldsymbol{H}w$, where $w = (w_j)$ is an N-dimensional positive weight vector satisfying $w_j \geq 0, \sum_{j=1}^{N} w_j = 1$ and $\mathbf{1}$ is the M-dimensional vector $(1, 1, ..., 1)$. Solving this regression for w and scalars α and β by writing it as an unconstrained nonlinear regression by transforming pointwise $w_j \to w_j^2$ to remove the positivity constraint, seeks breakpoints as a linear model of the entries in MIC histograms. For our data, the correlation coefficient of the resulting model was $\rho \approx 0.55$ whereby w has a sigmoidal shape that prioritises higher dosages over lower ones, this is to be expected because breakpoints concentrate around -1 to $4 \log_2 \mu g/ml$ (as can be seen in Fig 2).

These definitions lead to this question: where breakpoints have the greatest differences between EUCAST and CLSI, do their MIC distributions have the greatest differences? This question can be asked *within* both EUCAST and CLSI too but either way, we could find no support for these hypotheses, including in ATLAS.

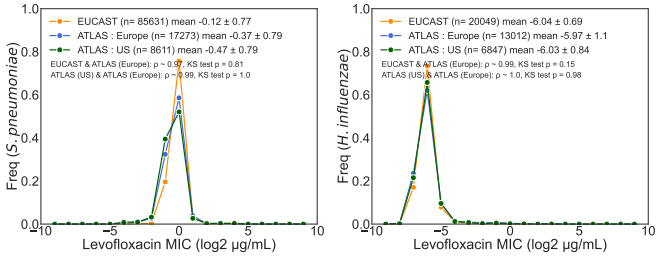

Fig 7 Two PA pairs with different CLSI and EUCAST breakpoints but very similar MIC data in US and Europe: Levofloxacin and (left) *S. pneumoniae* (right) *H. influenzae*.

Take Levofloxacin, it has the greatest mean breakpoint bias towards CLSI (Fig 4) but ATLAS provides no evidence of MIC distribution differences between US and EU as the Kolmogorov-Smirnoff test (KST) fails for every pathogen; Fig 7 show 2 example PA pairs of the many available. Here, *Streptococcus pneumoniae* and Levofloxacin have 3 MIC distributions, namely EUCAST, ATLAS (just for US data) and ATLAS (just European data) that are statistically 'indistinct' (by the KST) where the relevant $\log_2 \mu g/ml$ breakpoints are $(S, R) = (1, 3)$ for CLSI and $(S, R) = (-9.97, 1)$ for EUCAST. We contend that these breakpoints, relevant to the treatment of pneumonia, might be revisited based on the ATLAS data shown in Fig 7(left) which shows that the US and Europe have very similar MIC data, and, moreover, both are very similar to EUCASTs' own. A second striking example is *Haemophilus influenzae* and Levofloxacin whose breakpoints are $(S, R) = (1, 3.05)$ for CLSI and $(-4.06, -4.06)$ for EUCAST that is lower by over $5 \log_2 \mu g/mL$ units, yet European and US MIC histograms from EUCAST and ATLAS correlate strongly ($\rho > 0.99$, Fig 7(right)).

Results 4: local optimisation of a neural network ensemble to seek mechanism. We now ask this: what general principles or mechanisms lead to type I and II inconsistencies in breakpoints? To answer, we turned to artificial neural networks (ANNs) and trained 3,000 differently structured multi-layer, feedback and feed-forward ANNs to predict all EUCAST and CLSI breakpoints using EUCAST MIC data. We then chose the best performing $n = 100$ of these to form a decision ensemble (ANNE) that compromises between those n possibilities by taking the mean breakpoint decision after outlier removal. Typical correlation coefficients in ANNE between model predictions and target data are around $\rho \approx 0.8 - 0.85$ (data not shown).

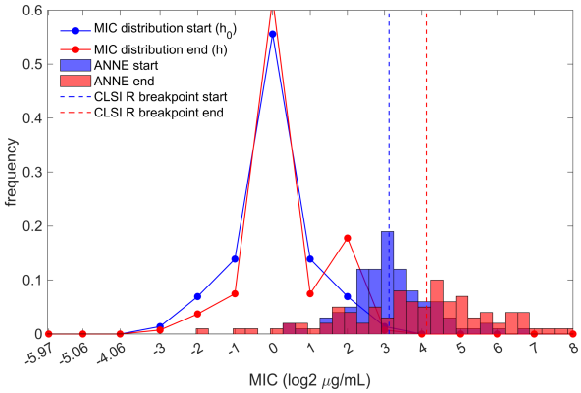

Fig 8 An iterative algorithm simulates breakpoint inconsistencies by starting at the unimodal blue MIC distribution, $\mathbf{h}_0$, estimating its R breakpoints using 100 neural nets in ANNE (blue histogram/dashed line) and then iteratively minimising the 'inconsistency ratio' $I(\mathbf{h})$ defined in the text. This algorithm ends at the red MIC distribution, $\mathbf{h}$, which is bimodal, whose variance of breakpoint decisions within ANNE (red histogram/dashed line) is greater than for $\mathbf{h}_0$.

Now, let $\mathbf{h}$ be an MIC distribution and let $\mathbf{h}_0$ be a (synthetic, i.e. not real world) unimodal MIC distribution. By reciprocating L_b we define an 'inconsistency ratio' $I(\mathbf{h}) := 1/L_b(\mathbf{h}, \mathbf{h}_0)$ whereby type I inconsistencies occur when $I(\mathbf{h})$ is 'small'. To determine inconsistencies, we applied the Nelder-Mead minimisation algorithm to $I(\mathbf{h})$, terminating it when the algorithm found a breakpoint 1-$\log_2$ MIC unit away from the ANNE-predicted breakpoint of $\mathbf{h}_0$ that we used as the starting point of this algorithm. This procedure yields a new, synthetic MIC distribution, $\mathbf{h}$, that Fig 8 illustrates as a red dotted line where $\mathbf{h}_0$ is a blue dotted line. Now, $\mathbf{h}$ is bimodal so, in terms of GMMs, this algorithm begins with a 1-cluster distribution and terminates at a 2-cluster distribution, worsening the quantitative measure of inconsistency, $I(\mathbf{h})$, en route. Interestingly, this change in MIC histogram is natural from an evolutionary perspective because it mirrors what happens when a genetic resistance mechanism spreads through a population of microbes. Although speculative, this analysis suggests a dynamic with a genetic basis can act, in theory, to increase uncertainty when setting breakpoints, as Fig 8 illustrates.

Finally, we used the ANNE to predict breakpoints for every EUCAST PA pair with at least 50 MICs (over 2,000, Fig 9). While the ANNE can set breakpoints, in principle, this leaves open the question of what an optimal breakpoint-setting mechanism might look like? Logistic regression has been proposed [7] for this when treatment outcomes are available and we propose that if this is not the case, our approach can help mitigate inconsistent breakpoints, using ANNEs to flag when new decisions are inconsistent with prior ones. Indeed, the correlation between US and European breakpoints decided by ANNE is high ($\rho \approx 0.94$).

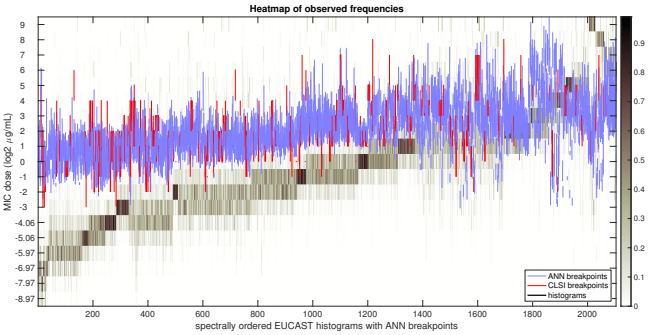

Fig 9 The analogy of Fig 2 for EUCAST data with at least 50 MICs, with breakpoints determined by ANNE.

Conclusion. We propose that optimal breakpoint classifiers could be found using variational techniques that determine breakpoint mappings, $b(\mathbf{h})$, by minimising a combination of $1/L_b$ and L_b subject to constraints imposed by data to mitigate type I and II inconsistencies. How to achieve this is, as yet, unclear and the subject of ongoing research.

This work is highly relevant clinically because we have shown important species, like ESKAPE pathogens, have breakpoints that seem inconsistent with their MICs. An important case are the Enterobacterales whose species all have (i) the same (or similar) within-continent breakpoints, (ii) different between-continent breakpoints and yet they have (iii) different ATLAS MICs between species whereas (iv) ATLAS MICs are consistent between continents; these 4 incongruent properties merit deeper investigation.

There are many flaws in our approach, some dictated by a lack of available data and the global coverage thereof. Biases in ATLAS might matter, including ones studied by us [8], so too our choice of metrics, or the relevance of statistical techniques, like Bayes PCA and KNN classifications of MIC data, which could turn out to be irrelevant to real-world clinical outcomes. Nevertheless, we still contend that ideas from Machine Learning (ML) could be incorporated into the decision pipelines of breakpoint-setting organisations. While neural nets trained on MICs alone cannot be a reliable way of placing breakpoints, it is surprising that ECOFFs are in use when GMMs provide far more structural insight into MIC distributions. If used in conjunction with ML techniques, like neural nets, GMMs and other modern statistical tools, it might well be possible to define breakpoints in a manner that is more quantitatively self-consistent than at present.

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