Evaluation of *Salmonella* resistance trends based on MICs mixture distributions in NARMS retail meat data

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Abstract

Monitoring and understanding trends in antimicrobial resistance among foodborne bacteria is vital to the identification of areas of concern and refining approaches to control antimicrobial resistance. Internationally accepted breakpoints are used to classify the antimicrobial susceptibility data (minimum inhibitory concentrations or MICs) into *susceptible, intermediate,* or *resistant* categories. The annual report of the National Antimicrobial Resistance Monitoring System (NARMS) employs a non-parametric statistical analysis of antimicrobial resistance trends based on these categories. This approach may exclude valuable information that would allow us to identify more subtle trends in MIC change. Here we employ a mixture distributions method that uses the full scale of MIC values to test the existence of significant *Salmonell*a resistance trends in the NARMS retail meat data collected between 2002 and 2019. Through this method, we investigated potential trends using MIC data including timepoints before and after the implementation of FDA guidance for industry GFI #209 and GFI #213 fully implemented by 2015, which led to the voluntary removal of growth promotion claims from medically important antimicrobials intended for use in food animals. The results showed an overall decrease in *Salmonella* MICs against all antimicrobials tested with an estimated 11.11% minimum decrease. Although we cannot solely attribute the observed decrease in *Salmonella* resistance levels to GFI #209 and GFI #213, we believe the implementation of these FDA guidance documents may have played a role given the timing of such significant changes.

Introduction

- Monitoring trends in resistance is an important public health function for detecting the development of resistance, planning targeted interventions and assessing the effects of such interventions
- Based on epidemiological or clinical cut-off values the distributions of minimum inhibitory concentrations (MIC) among isolates tested for antimicrobial susceptibility are dichotomized into groups of 'non-resistant' vs. 'resistant' for resistance interpretation.
- Dichotomizing the full scale of MIC values also leads to some loss of information and some trends might not be detectable on the collapsed scale.
- FDA issued 2 guidance, GFI #209 & GFI 213, fully implemented by 2015 and may affect the current antimicrobial resistance trends.

Material and Methods

MIC values represent a mixture of two distributions $D_1$and D_2 of susceptible and resistance isolates with probability p_1 and p_2 respectively. The overall probability density function $v(x)$ of MIC can be written as: $v(x) = (1-v)f(x) + v * g(x)$
where: $f(x)$denotes the density associated with the distribution D_1 of MICs from susceptible isolates and $g(x)$ denote the density associated with the distribution D_2 of MICs from resistance isolates. Considering a simple linear regression $y_{ij} = \beta_0 + \beta_1 t_i + \epsilon_{ij}$ where ϵ_{ij} is normally distributed with mean 0 and variance σ^2. Time effect fit results in time dependent mean: $\mu(t) = \beta_0 + \beta_1 t$. Given the mixture distributions aspect of the data $\mu(t) = ((1-v(t))\mu_1) + v(t)\mu_2$, where $v(t)$ is the true proportion of resistance isolates at time t. $v(t) = \frac{\mu(t)-\mu_1}{\mu_2-\mu_1}$, where μ_1 is the true MIC mean of non-resistance isolates, and μ_2 is the true MIC mean of resistance isolates.

Estimation and hypothesis testing

- Density estimation methods are used to estimate μ_1 and μ_2
- Least squares regression methods are used to estimate $\mu(t)$
- The proportion of resistance isolates $v(t)$ at time t can be estimated as: $\hat{v}(t) = \frac{\hat{\mu}(t)-\hat{\mu}_1}{\hat{\mu}_2-\hat{\mu}_1}$

Testing a linear trend
the null hypothesis:
$H_0: v(t) = c$, where c is a constant
vs $H_A: v(t) > c$ or $H_A: v(t) < c$

- Equivalent to testing:

$H_0: \beta_1 = 0$ vs
$H_A: \beta_1 > 0$ or $H_A: \beta_1 < 0$

Percent change (increase or decrease) = [(new value - original value)/original value] * 100

Figure 1: From left to right, the distribution of MICs in *Salmonella* isolates susceptibility tested against amoxicillin, ampicillin, ceftriaxone, and cefoxitin in chicken parts, ground turkey, ground beef, and pork chops combined between 2002-2019. A larger dot corresponds to a high proportion of isolates with this MIC for a particular year.

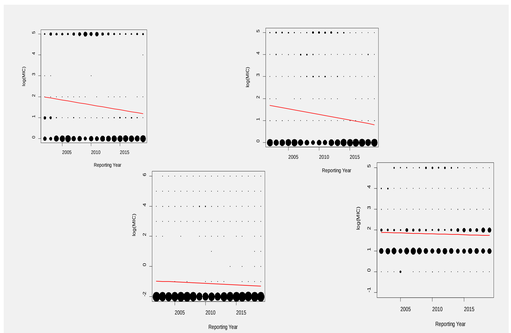

Figure 1: The red lines represent the fitted linear trend over time for amoxicillin, ampicillin, ceftriaxone, and cefoxitin, the 4 antimicrobials with the fastest decrease in MIC between 2015 and 2019. Namely, 1566.7 %, 666.67%, 253.84%, 133.33% for amoxicillin, ampicillin, ceftriaxone, and cefoxitin, respectively.

Results and Discussions

Table 1: On log 2 scale, estimated true MIC means (μ), variances (σ), and mixing proportions (v) based on the mixture distribution model of *Salmonella* isolates from retail meats tested against gentamicin, ceftriaxone, ampicillin, chloramphenicol, sulfonamides, tetracycline, streptomycin, trimethoprim–sulfamethoxazole, amoxicillin, cefoxitin.

Antimicrobial		μ	σ	v
Gentamicin	Susceptible	-1.45	1.11	0.88
	Resistance	6.09	0.40	0.12
Ceftriaxone	Susceptible	-10.27	3.26	0.86
	Resistance	4.06	1.07	0.14
Ampicillin	Susceptible	-1.34	1.51	0.71
	Resistance	6.64	0.24	0.29
Chloramphenicol	Susceptible	2.54	0.53	0.97
	Resistance	7.92	0.52	0.03
Sulfonamides	Susceptible	-0.171	2.91	0.31
	Resistance	1.98	0.002	0.69
Tetracycline	Susceptible	-1.00	0.50	0.25
	Resistance	39.35	86.15	0.75
Streptomycin	Susceptible	2.48	0.78	0.18
	Resistance	5.52	0.96	0.82
Trimethoprim–Sulfamethoxazole	Susceptible	-6.55	2.15	0.98
	Resistance	14.59	2.07	0.12
Amoxicillin-Clavulanic Acid	Susceptible	-6.59	1.13	0.63
	Resistance	3.88	2.62	0.37
Cefoxitin	Susceptible	1.30	0.51	0.78
	Resistance	4.59	3.90	0.22

Figure 2: This graph is a representation of a mixture of normal distributions fitted on *Salmonella* isolates tested against cefoxitin, where μ and σ MICs of susceptible isolates are 1.30 and 0.51 respectively and those for resistance isolates are 4.59 and 3.9, on log2 scale .

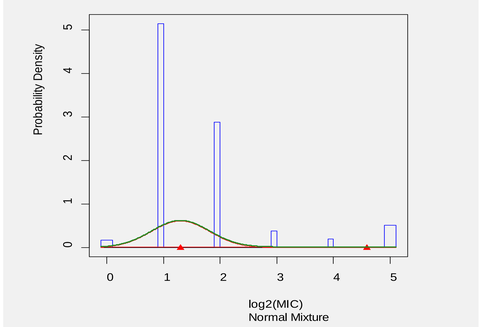

Table 2. Estimates, on log2 scale, of the slope (and its standard error) of the fitted trend lines overtime, p-value, and percent change (decrease/increase in MIC) for the period of 2002 -2015 and 2002-2019

	2002-2015			2002-2019			
Antimicrobial	**Slope**	**Standard error**	**P-value**	**Slope**	**Standard error**	**P-value**	**%change**
Gentamicin	β_1 =-0.008	0.007	0.290	β_1 =-0.0083	0.004	0.054	-0.0083 is a 3.75% decrease of -0.008
Ceftriaxone	$\beta_1 = 0.013$	0.008	0.133	β_1 =-0.020	0.005	<.001	-0.002 is a 253.84% decrease of 0.013
Ampicillin	β_1 =-0.006	0.008	0.461	β_1 =-0.046	0.005	<.001	-0.046 is a 666.67% decrease of -0.006.
Chloramphenicol	β_1 =-0.017	0.002	<.001	β_1 =-0.005	0.001	<.001	-0.005 is a 70.588% increase of -0.017
Sulfonamide	$\beta_1 = -.030$	0.005	<.001	$\beta_1 = -.042$	0.003	<.001	-0.042 is a 40% decrease of -0.03.
Tetracycline	$\beta_1 = 0.013$	0.005	0.024	$\beta_1 = -.008$	0.003	0.031	-0.008 is a 106.15% decrease of 0.13.
Streptomycin	$\beta_1 = -0.084$	0.003	<.001	$\beta_1 = -0.105$	0.002	<.001	-0.105 is a 25% decrease of -0.084
Trimethoprim–Sulfamethoxazole	$\beta_1 = -0.009$	0.001	<.001	$\beta_1 = 0.008$	0.001	<.001	-0.008 is a 11.111% increase of -0.009.
Amoxicillin-Clavulanic Acid	$\beta_1 = -0.003$	0.007	0.638	$\beta_1 = -0.05$	0.004	<.001	-0.05 is a 1566.7% decrease of -0.003.
Cefoxitin	$\beta_1 = 0.024$	0.005	<.001	$\beta_1 = -0.008$	0.003	0.005	-0.008 is a 133.33% decrease of 0.024.

Which is the leading commodity in decrease of resistance in MIC to each antimicrobial tested?

- The leading commodity for gentamicin is ground turkey with **380%** decrease faster in 2019 as compared to 2015
- The leading commodity for Ceftriaxone is chicken parts with **116%** decrease faster in 2019 as compared to 2015
- The leading commodity for Ampicillin is pork chops with **322.22%** decrease faster in 2019 as compared to 2015
- The leading commodity for Chloramphenicol is ground beef with **190.91%** decrease faster in 2019 as compared to 2015
  - ➢ Ground beef is the only commodity with decreasing MIC
- The leading commodity for Tetracycline is Ground turkey with **500%** decrease faster in 2019 as compared to 2015
- The leading commodity for Streptomycin is Ground beef with **119.15%** decrease faster in 2019 as compared to 2015
- The leading commodity for Trimethoprim–Sulfamethoxazole is Ground beef with **20%** decrease faster in 2019 as compared to 2015
- The leading commodity for Amoxicillin is chicken parts with **116%** decrease faster in 2019 as compared to 2015
- The leading commodity for Cefoxitin is chicken parts with **860%** decrease faster in 2019 as compared to 2015

Conclusion

This approach exploits the continuous nature of the MIC data. Limitations: if there is no clear separation between the two populations (resistance and susceptible), the method may not work. It's still under study on how the method can be used to test trends in resistance to multiple drugs. FDA guideline GFI 209 and GFI 213 may not be the only contributor to the decreasing trends observed in this study, a look into antimicrobials use data may shade light into this.