Identification of distribution components from antibiotic resistance data - Opportunities and challenges

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During the 20th century, antibacterial agents have made modern medicine possible. However, the dramatic increase of resistant and multiresistant bacterial pathogens is now widely recognized as a global challenge for human health (Roca et al. 2015; Antimicrobial Resistance 2016; ECDC 2017).

The mechanisms of antibiotic resistance are diverse and a large number of antibiotic resistance genes is known today. Crucially, many resistance genes can be found on mobile genetic elements such as, for example, plasmids. The latter can not only be transmitted between individuals of the same strain but even between different species. Big efforts have been made to understand the processes of lateral gene transfer in a medical context, but there is still lack of understanding how environmental factors influence evolution and transmission of antibiotic resistances. Therefore, "global efforts are required to characterize and quantify antibiotic resistance in the environment" (Berendonk et al. 2015).

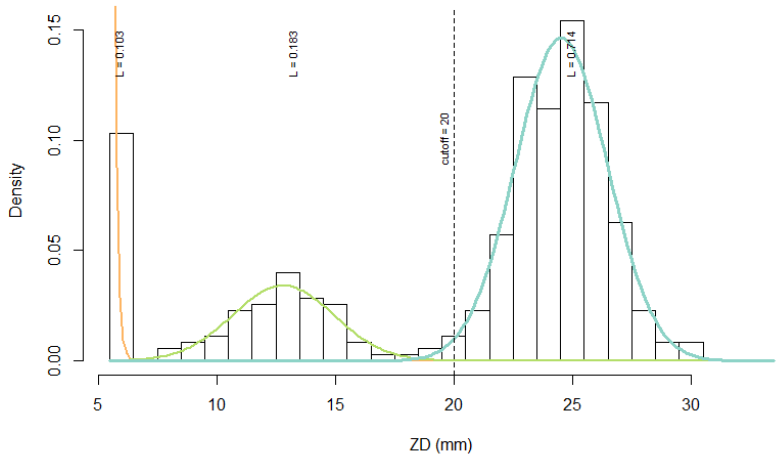

Fig 1: Zone diameter distribution with resistant (orange), intermediate (green) and wild-type sub-population (blue), L = proportions of the components, cutoff = 1% quantile of the wild type.

Phenotypic antibiotic resistance is generally studied in growth experiments where bacterial isolates are cultivated under drug exposure. This is done either in liquid trials to identify the minimum inhibitory concentration (MIC), or as so-called diffusion test on an agar plate, where the diameter of inhibition (zone diameter, ZD) is recorded. In order to identify the level of antibiotic resistance in a whole population, the MIC or ZD is recorded for hundreds or thousands of isolates. The obtained MIC or ZD values for each antibiotic form then a univariate, multi-modal distribution (Fig. 1).

Environmental populations can be composed of different geno- and phenotypes (Fig 1):

1. a resistant sub-population composed of strains that still grow on high antibiotic concentrations,
2. an intermediate sub-population whose strains tolerate moderate levels of antibiotics,
3. a so-called "wild type" denoting strains that are antibiotic-sensitive, i.e. not resistant.

The analysis aims to identify parameters (location, variance, quantiles) of these sub-populations, even if the distributions overlap. One important measure is the ECOFF (epidemiological cutoff) value, a quantile that separates the wild type from intermediate and resistant strains. In reality, MIC or ZD values are subject to a varying number of components with possible overlaps between sub-populations. Moreover, spurious distribution components may show up due to coarseness of the data.

It is widely accepted that the sub-populations' proportions (e.g. resistant and susceptible) are controlled by selection processes, e.g. a higher proportion of resistant bacteria in the waste water effluent of hospitals or animal farms. By contrast, it is usually taken for granted that the statistical characteristics of the wild type are universal world wide. In practice, parameters of wild type distributions differ between studies, but such variations were considered as methodological variation (laboratory effect). However, we recently analyzed ZD values of sewage and river-borne *Escherichia coli* isolates and compared them with each other, and with publicly available data (EUCAST 2018). Our results indicate that location and quantile shifts of the wild-type component cannot be attributed to laboratory effects alone. We hypothesize that selection processes in natural samples occur at a sub-acute level (low antibiotic concentrations affecting intermediate and wild-type sub-populations), that can eventually lead to an evolution of resistance.

The presentation will focus on the identification of distribution components from inhibition zone diameter data obtained from agar diffusion tests. Heuristic methods will be discussed and a maximum likelihood approch is introduced. Promising outputs of the existing approaches will be presented along with remaining challenges: robust estimation of the initial guess, reduced influence of binning, avoidance of redundant distribution components, choice of identification approaches (MLE, EM, Bayesian, etc), and application to MIC data with relatively coarse scales.

Berendonk, Thomas U., Célia M. Manaia, Christophe Merlin, Despo Fatta-Kassinos, Eddie Cytryn, Fiona Walsh, Helmut Bürgmann, et al. (2015) Tackling Antibiotic Resistance: The Environmental Framework. Nature Reviews Microbiology 13: 310. doi:10.1038/nrmicro3439.

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