Opinion: Quantitative Antibacterial Disk Susceptibility Results What are they Good For?

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The main function of *in vitro* susceptibility testing is to provide the clinician with a tool to choose the antibacterial compound with the best odds of therapeutic success. The predictive value of the test, i.e. the likelihood of detecting the most appropriate drug to overcome the infection, is of paramount importance. Unfortunately, the predictive value may be limited due to a number of factors. These factors may be divided in two groups: 1. those stemming from differences between *in vivo* and *in vitro* conditions and 2. those stemming from the conversion of the results expressed as inhibition zone (IZ) diameter to minimal inhibitory concentration (MIC), a method that has a statistical significance rather than a biological accuracy. Interestingly there are only limited publications relating inhibition zones to therapeutic outcome. Several reports have shown such a correlation (1, 2, 3), although it seems that this may not be true for some microorganisms such as *Pseudomonas aeruginosa*.

DIFFERENCES BETWEEN *IN VIVO* AND *IN VITRO* CONDITIONS

Under *in vitro* conditions the entire quantity of the antibacterial compound interacts with the microbe. This may be not so under *in vivo* conditions where the microbe may be exposed to a lower concentration of the drug due to pharmacodynamic and/or pharmacokinetic considerations (4), such as its binding to various tissues or molecules, lack of penetration to an anatomic compartment or inactivation by products of the inflammatory process, such as pus. Moreover, the physiology and anatomy of the target organ may differ from that on which the drug was tested to determine achievable concentrations. Other factors that may influence the *in vivo* activity of the drug are the individual variations in the physiology of the treated animal, a consideration that has given rise to the new discipline of pharmacogenetics (5).

The nature of the interaction of the compound with the microorganism may also play a role. *In vitro* test assesses the susceptibility of a single colony to an antibacterial agent. In opposition, in the host, the drug has to act often in sites colonized by a large variety of microorganisms, interacting by various means such as quorum sensing (a phenomenon by which gene expression is induced by population density). Microbial aggregates, organized in biofilms, may pose additional therapeutic difficulties stemming from factors such as diminished diffusion and resistant subpopulations typically present in the deeper layers of such consortia (6). Moreover, it has been shown that the susceptibility of the bacterial population of the same species, isolated from a given specimen, may vary (4). Thus the colony, upon which the susceptibility test is performed, usually chosen randomly and which may not be representative, may give misleading results.

Other, less troublesome, cases of discrepancy between *in vitro* and *in vivo* results are those in which a drug to which the relevant bacterium was found resistant *in vitro* appears to have successfully treated the infection. In fact, instead of the near to 100% failures expected in such cases, the observed rate may be significantly lower (1). This may be in some cases not related to the antimicrobial therapy but rather due natural body defenses overcoming the infection or removal of the underlying cause.

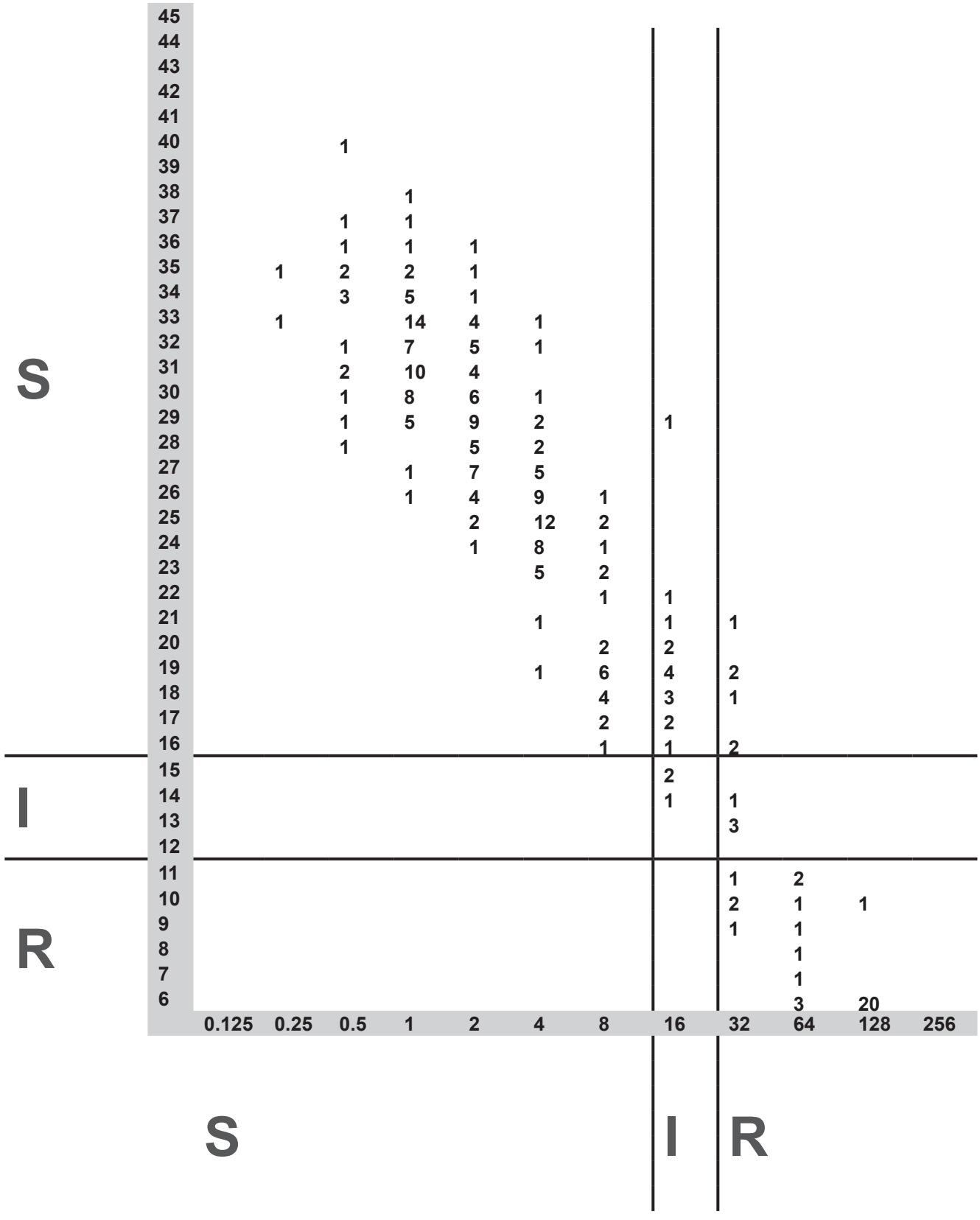

Figure 1. A hypothetical scatterplot associating minimal inhibition concentrations (μg/ml, X axis) to inhibition zones (millimeters, Y axis). S: susceptible, I: intermediate, R: resistant

INHIBITION ZONE TO MIC CONVERSION

While methods to determine *in vitro* MIC values are becoming more and more common in large laboratories, the price of the hardware and disposables required to perform the test remain prohibitive for smaller laboratories. Consequently, the less expensive disk diffusion susceptibility test is still often performed in veterinary laboratories. To convert the measured IZ diameter to MIC, both values are measured for a large number of strains of a given bacterium. A regression line is calculated from the results and the conversion is carried out using the error-rate bounded method (7). As a rule, the measured value of IZ for a given MIC are not uniform but are distributed through a range of values, with most observations converging around the mean. A similar phenomenon is observed for the MIC values for a given IZ (Figure 1). The wider the spread the greater the approximation of the regression line and therefore the uncertainty and inaccuracy of the conversion for a given value. The theoretical scatterplot shown in Figure 1 is based on other such plots (4, 7, 8). It shows the association between IZ and MIC and is divided into areas by the breakpoints which define a bacterium as resistant, intermediate or susceptible to a given antibacterial drug. The breakpoints are determined by methods beyond the scope of this article. These areas represent all the possibilities of combinations between resistant, intermediate and susceptible for MIC and those for IZ, i.e. nine (3^2) areas. If no intermediate values are defined the areas are reduced to four (2^2). Most observations have to be located in the areas of consensus between the methods. However, due to the above-described method of conversion, some results may be located in areas representing an error. The most severe error is the interpretation of an IZ result as susceptible while by its MIC the microbe is resistant (upper right square in Figure 1), since it may lead to an attempt of therapy with an unsuitable drug (8).

Finally, the human factor and technical errors may constitute additional sources of discrepancies between *in vitro* and *in vivo* results. The introduction of computerized measuring systems during the last years has contributed to reduce some of these errors (9).

THE IMPORTANCE OF QUANTITATIVE RESULTS

Usually the results of disk susceptibility tests are recorded quantitatively but reported qualitatively, i.e. as susceptible, intermediate or resistant, according to accepted standards, such as that of the CLSI (10). Arguably, there may be two reasons to provide the clinician with quantitative data.

1. By qualitative criteria alone all inhibition zones equal or larger than 16 millimeters, based on a scatterplot such as shown in Figure 1, will be interpreted as susceptible. However, at the minimum inhibition zone of 16 millimeters, 2 out of 4 examined strains would be regarded as susceptible while by their MIC results they are resistant; at higher IZ values, this risk diminishes and then disappears.
2. Another hypothetical reason to report the susceptibility quantitatively is related to the "hurdles" the drug has to overcome in the host before it can act on the microbe, as described above. Since the compound in the disk creates a gradient by diffusion, the larger the IZ, the lower the concentration that inhibits the bacterium's growth. Thus, if the IZ is large, the bacterium is more susceptible and consequently the probability that the drug will reach it in effective concentrations, in the host, is higher.

Breakpoints vary according to bacterium-antibiotic-host combinations and distinct drugs behave differently on the disk diffusion test medium. Consequently the interpretation of IZ size vary and a reference value has to be provided; the breakpoint for susceptibility being the best candidate. A ratio of the measured IZ to the breakpoint for susceptibility (Susceptibility Coefficient) may provide the value and the reference point at the same time. A high ratio indicates that the IZ measured in a test is way beyond the susceptible breakpoint. It means that the bacterium is more susceptible to the tested drug and that the chances of an error are lower and thus the predictive value of the test is higher.

The significance of the quantitative susceptibility results must be made clear to the practitioners: they are an indication, an additional tool to choose the right drug, and by no means to be taken as a mathematical certainty, especially when the differences between two results are relatively small.

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