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# Interpretation of Antibacterial Susceptibility Reports

## *In Vitro* versus Clinical Break-Points

*Guy W. Amsden* and *Janelle M. Duran*

Clinical Pharmacology Research Center, Bassett Healthcare, Cooperstown, New York, USA

**Abstract**

Currently, antibacterial activity is measured primarily via *in vitro* laboratory tests. Clinicians rely heavily upon the reported susceptibility gained via *in vitro* laboratory tests when choosing an antibacterial agent. An evolving concept is to utilise pharmacodynamic and pharmacokinetic drug properties in addition to *in vitro* susceptibility reports to assess the potential effectiveness of an antibacterial agent against a specific pathogen. This article presents examples of the utility of these concepts in terms of optimal clinical use of common antibacterials as well as more informed interpretation of the *in vitro* literature.

For years antibacterial susceptibility has been measured utilising *in vitro* tests such as antibacterial impregnated disks, broth microdilution, and more recently via E-test strips. These *in vitro* tests provide a simple numerical limit by which antibacterial activity *in vivo* is estimated. The numerical limit attained through these tests, the minimum inhibitory concentration (MIC), is compared with the serum/plasma concentrations achieved with commonly used dosages of a drug in order to predict an antibacterial agent's likelihood for clinical and microbiological success. Based on these data, actual MIC break-points are determined so that depending on the MIC of a pathogen, the result for a specific antibacterial can be reported to the clinician as either susceptible, intermediately susceptible/resistant, or resistant. Although this practice takes into account the most basic of antibacterial activity properties, by making sure that the concentrations that will be achieved with an average dose will be above the MIC of the pathogen, it does not take into account the pharmacokinetics of many types of agents or the pharmacodynamics associated with each class of agents.

This opinion article details some of the difficulties of utilising *in vitro* data blindly without taking into account the pharmacokinetic and pharmacodynamic properties of an antibacterial agent. Although potentially unpopular, it is necessary to utilise these evolving concepts in deciding antibacterial choice and dosage regimens in this day of increasing pathogen resistance.

### 1. Antibacterial Pharmacokinetics and Pharmacodynamics

The most commonly used antibacterials in this day and age include the β-lactams, aminoglycosides, fluoroquinolones and macrolides. Each of these classes of drugs is handled differently by the human body and each has different ways by which it should be utilised to be effective against a given pathogen. β-lactams do not penetrate host cells to any significant extent and distribute evenly throughout the body, with the exception of the central nervous system. As a result, it is possible to measure a serum concentration at a given time-point after a dose has been administered and have it be relatively reflective of the concentration in the

extracellular space of any perfused organ or tissue site. Aminoglycosides have similar properties to β-lactams, with the exception that they can be taken up by host cells over time with continued administration. However, this cellular uptake is usually associated with toxicity rather than efficacy. Macrolides and fluoroquinolones differ markedly from β-lactams and aminoglycosides, but are very similar in their distribution properties to each other. Both of these classes of compounds have extensive distribution throughout the body. Although their serum/plasma concentrations sometimes appear low, or even sub-therapeutic, their concentrations at infection sites are up to a log-fold higher than the corresponding blood concentrations. Beyond the tissue sites, both classes are taken up avidly by white blood cells (phagocytes), which are responsible for the clearing of the pathogen, regardless of whether it is in a tissue site or in the bloodstream. These intracellular concentrations are even higher than the tissue sites and can be up to 2 log-folds higher than corresponding blood concentrations.[1,2]

Understanding the pharmacokinetics of these classes of agents is only half the picture. The other part that is key to the proper interpretation of susceptibility data is the pharmacodynamic properties of the agents. β-lactams and macrolides are time-dependent, or concentration-independent, killers.[3] The key to using them successfully is to keep the concentrations of the agent at the site of pathogen clearance above the MIC for as long as possible during the dosage interval.[4] In contrast, aminoglycosides and fluoroquinolones are concentration-dependent, or time-independent, killers. With these classes of drugs it is important to achieve a certain peak concentration to MIC ratio with each dose to achieve an optimal effect against the pathogen. With aminoglycosides, a ratio of 10 : 1 is thought to be ideal and for fluoroquinolones a ratio of approximately 12 : 1 appears to be optimal.[5,6] One pharmacodynamic concept that ties all the classes together is trying to optimise the area under the serum concentration time curve ($AUC_{0-24}$) to MIC ratio ($AUC_{0-24}$ : MIC). It has been suggested that it is necessary to achieve a ratio of at least 125 $SIT^{-1}h$ (where SIT is serum inhibitory titre) for Gram-negative pathogens, but that the needed ratio for optimal activity against Gram-positive organisms may be as low as 30 $SIT^{-1}h$.[7,8]

## 2. Interpretation and Application of Susceptibility Data

As stated in the opening paragraph, an MIC is a reflection of the concentration of drug that needs to be exceeded to achieve optimal outcome. This concept supposes that the concentrations that pathogens will be exposed to at the infection site are always equal to those achieved in the blood. However, as discussed in section 1, this is not true for all classes of antibacterials and the extrapolation of this concept to all classes of antibacterials is naïve. Additional extrapolation, that all areas in the body will have equilibrium-based exposure is also naïve as we know that many classes of antibacterials, including aminoglycosides, fluoroquinolones and β-lactams, are concentrated to a much higher degree in the urinary tract than in the blood.

There is no doubt that the break-points that are decided upon by national and international advisory boards are helpful in guiding the use of antibacterials but these guidelines sometimes incorporate clinically unrealistic break-points and do not take into account the pharmacokinetic properties of many of the newer agents. As an example, the National Committee for Clinical Laboratory Standards (NCCLS) guidelines state that a MIC for *Pseudomonas aeruginosa* to gentamicin of 4.0 mg/L is considered susceptible.[9] Although concentrations well above a 10 : 1 ratio are easily achieved in the urinary tract, based on the pharmacodynamic data that is available it would be necessary to achieve a post-distributional peak serum gentamicin concentration of 40.0 mg/L for infections involving other tissue sites, which is clinically unrealistic as well as potentially hazardous. New concepts involving once daily administration of aminoglycosides may achieve peak concentrations that approach this magnitude; however, their safety and efficacy in a seriously ill population that is not otherwise healthy

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and requires prolonged antibacterial treatment has yet to be fully elucidated.[10]

Another example would be the interpretation of macrolide activity against sensitive and resistant pneumococci. Standard thinking would indicate that although the serum concentrations achieved with clarithromycin (1 to 2 mg/L) should be active the vast majority of times against sensitive isolates ($MIC_{90}$ 0.125 mg/L), azithromycin should inherently fail in the majority of instances because of its low serum concentrations of approximately 0.4 mg/L that stay above the pneumococcal $MIC_{90}$ of 0.25 mg/L for barely any of the dosage interval, much less any of the washout period. Although clarithromycin has demonstrated the expected good activity, azithromycin also has good clinical and microbiological efficacy, contrary to what should be believed from serum concentrations. This good activity most likely has little to do with the serum concentrations of the drug but rather the high infection site concentrations and even higher phagocyte concentrations (clarithromycin ≈20 mg/L; azithromycin ≈80 to 100 mg/L). Because of these factors, not only do these drugs have good activity against sensitive pneumococcal isolates but also potentially against isolates resistant as a result of an efflux mechanism. Although national laboratory standards have taken the kinetics of these drugs into account somewhat more than with others, chances are that their clinical break-points may be a little higher than those of the current laboratory standards (resistance = MIC >1.0 mg/L for clarithromycin and >2.0 mg/L for azithromycin). If true, then the complete disparity between the lack of large numbers of clinical failures despite widespread reports of increasing *in vitro* resistance may be more easily explained.[11]

One of the most common fallacies when interpreting pneumococcal resistance literature with regard to β-lactams is that it would be foolhardy to attempt to use earlier generation agents in patients with pneumococcal infections. Despite literature reports of several countries throughout the world having penicillin resistance rates of at least 50%, it is interesting to note that many of these countries still use β-lactams with great success. One of the reasons is the way that resistance data are reported for these drugs and this pathogen. Pneumococcal break-point standards for penicillin and aminopenicillins (i.e. amoxicillin, ampicillin) state that a strain is sensitive if the MIC is <0.1 mg/L, intermediately sensitive/resistant with an MIC of 0.1 to 2.0 mg/L, and resistant (or highly resistant) if the MIC is >2.0 mg/L. The vast majority of reports group the latter 2 categories together and quote the sum of them as the percentage of isolates that are resistant. In reality, the incidence of truly resistant isolates is much smaller than the composite number leads the reader to believe, and in fact the incidence of isolates with an MIC over 4.0 mg/L is relatively rare.

The other reason is that we have learned how to use these agents to optimise their dynamics against the pathogen. The first large scale examples of this were studies in adults by Pallares et al.[12] and in children by Friedland[13] in which all patients with pneumococcal infections, regardless of sensitivity, were treated with high dosages (ampicillin 2g every 6 hours, penicillin 2MU every 4 hours) of β-lactams. Despite conducting these studies in areas associated with a high incidence of resistant pneumococci, mortality rates were not significantly different between those patients treated for a sensitive isolate and those treated for a resistant isolate. Rather than switch classes or generations of agents because of the resistance reports, the higher dosages of the drugs used maintained concentrations above 2 mg/L for at least 80% of the dosage interval.

Based on these data it has been suggested that the definition of resistance to these drugs be altered by honing the definition to 2 categories rather than 3. By doing this, an isolate would be considered resistant to penicillin/aminopenicillins only if the MIC were above 2.0 mg/L. All others would be considered sensitive. Additional confidence in this concept has recently been published as otitis media treatment guidelines from the Centers for Disease Control and a Drug Resistant *Streptococcus pneumoniae* Working Group. These guidelines instruct clinicians to double the current starting dosage of

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amoxicillin from 40 to 45 mg/kg/day to 80 to 90 mg/kg/day, which in effect incorporates the conclusions from the previously described studies.[14] By utilising these concepts for otitis as well as other types of pneumococcal infections, a large majority of pneumococcal isolates, including those defined as intermediately susceptible/resistant, should still be susceptible to the effects of these earlier generation and cheaper agents.

A final example of confusion over interpretation of data involves the practice of reporting susceptibility results merely with an S, I or R to denote susceptible, intermediate and resistant, respectively. In many instances utilisation of these types of results would incur no negative clinical impact, but in some it can. An MIC of 1.0 mg/L for ciprofloxacin to either *S. pneumoniae* or *P. aeruginosa* is considered susceptible. However, if the pharmacodynamic concepts that have been proposed for optimising quinolone activity are true, then the activity against isolates with this MIC should be significantly impaired. Even with an oral regimen of 750mg twice daily, a 24-hour AUC would be only 32 mg/L · h and the maximum concentration ($C_{max}$) 4.0 mg/L. By comparing the MIC to both of these kinetic measures, it is quite obvious that even a high oral regimen of ciprofloxacin does not nearly achieve an AUC:MIC of 125 $SIT^{-1}h$, or a peak $C_{max}$ : MIC of 10-12:1.

## 3. Conclusions

The ability to compare an MIC value with a serum concentration has served clinicians well for a number of years. However, with the introduction of new classes of antibacterials and the increasing incidence of resistance, interpretation of these standardised break-points in terms of choosing an antibacterial for a specific patient with a specific infection/pathogen is becoming more difficult. Taking the pharmacokinetics and pharmacodynamics of antibacterials into the decision-making practices of patient treatment as well as true break-point determination will allow us to not only treat patients optimally but also better enable us to interpret the *in vitro* literature appropriately. Additionally, knowing the *in vivo* properties of antibacterials will allow clinicians to interpret the *in vitro* resistance and decide for themselves the actual point at which a specific antibacterial agent may no longer be effective.

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Correspondence and offprints: Dr *Guy W. Amsden*, Clinical Pharmacology Research Center, Bassett Healthcare, One Atwell Road, Cooperstown, NY13326, USA.
E-mail: guy.amsden@bassett.org

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