A Long Journey from Minimum Inhibitory Concentration Testing to Clinically Predictive Breakpoints: Deterministic and Probabilistic Approaches in Deriving Breakpoints

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Abstract

Since the origin of an "International Collaborative Study on Antibiotic Sensitivity Testing" in 1971, considerable advancement has been made to standardize clinical susceptibility testing procedures of antimicrobial agents. However, a consensus on the methods to be used and interpretive criteria was not reached, so the results of susceptibility testing were discrepant. Recently, the European Committee on Antimicrobial Susceptibility Testing achieved a harmonization of existing methods for susceptibility testing and now co-ordinates the process for setting breakpoints.

Previously, breakpoints were set by adjusting the mean pharmacokinetic parameters derived from healthy volunteers to the susceptibilities of a population of potential pathogens expressed as the mean minimum inhibitory concentration (MIC) or MIC90%. Breakpoints derived by the deterministic approach tend to be too high, since this procedure does not take the variabilities of drug exposure and the susceptibility patterns into account. Therefore, first-step mutants or borderline susceptible bacteria may be considered as fully susceptible. As the drug exposure of such sub-populations is inadequate, resistance development will increase and eradication rates will decrease, resulting in clinical failure.

The science of pharmacokinetics/pharmacodynamics integrates all possible drug exposures for standard dosage regimens and all MIC values likely to be found for the clinical isolates into the breakpoint definitions. Ideally, the data sets used originate from patients suffering from the disease to be treated. Probability density functions for both the pharmacokinetic and microbiological variables are determined, and a large number of MIC/drug exposure scenarios are calculated. Therefore, this method is defined as the probabilistic approach. The breakpoints thus derived are lower than the ones defined deterministically, as the entire range of probable drug exposures from low to high is modeled. Therefore, the amplification of drug-resistant sub-populations will be reduced.

It has been a long journey since the first attempts in 1971 to define breakpoints. Clearly, this implies that none of the various approaches is right or wrong, and that the different approaches reflect different philosophies and mirror the tremendous progress made in the understanding of the pharmacodynamic properties of different classes of antimicrobials.

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Introduction

Since the early days of antibiotic therapy, indicators have been sought that could support appropriate dosing regimens. With the introduction of sulfonamides in 1935 and penicillin in 1942, data from preclinical studies and indicators of clinical success or failure were used to ascertain dose and dosing interval selection.

The microbiological parameter used since the beginning of antibiotic therapy is the minimal inhibitory concentration (MIC) of the antimicrobial agent for the pathogen. Initially, the results of MIC testing varied significantly because of the different materials and methods used, so, in 1971, an ''International Collaborative Study on Antibiotic Sensitivity Testing (ICS)'' [1] addressed important aspects of the standardization of MIC testing.

Furthermore, this group attempted to simplify the cumbersome and time-consuming MIC testing by replacing serial dilutions over a broad range of concentrations with one or more critical concentrations that separated MIC distributions into two sub-populations, i.e., a drug-susceptible wild-type sub-population (in which acquired or mutational resistance mechanisms are absent) and a drug-resistant sub-population (strains harboring one or more resistance determinants). This simplified method

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used to separate organisms into ''resistant'' and ''susceptible'' categories is now commonly referred to as the ''breakpoint'' technique, a term first used in the report of this study group [1]. Thus, the original definition of a ''breakpoint'' was a drug concentration that differentiated two bacterial sub-populations. The breakpoint technology applied in the antimicrobial susceptibility test systems is still used in this sense.

On the one hand, this report is a hallmark in the methodology of performing MIC tests and in the interpretation of susceptibility testing; on the other hand, however, the views of the participants in this study differed too much to reach a consensus on the meaning of the classifications ''susceptible'' and ''resistant.'' Some used the definitions in a microbiological sense to differentiate between two distinct populations, i.e., those with the absence or presence of resistance mechanisms, whereas others used the definitions in a clinical context in order to guide therapy.

Consequently, a number of mechanisms and philosophies exist by which the breakpoint between a susceptible and resistant population may be established. In the USA, the ''National Committee for Clinical Laboratory Standards'' (NCCLS) formerly and nowadays the ''Clinical and Laboratory Standards Institute'' (CLSI) publishes such standards [2, 3]. These guidelines and definitions are used in many parts of the world. In Europe, however, several national societies and committees have different philosophies and recommend different methods for susceptibility testing; and to complicate the matter further, different national authorities in the EU have, in the past, granted licenses for different doses of the same antibacterial agent.

As a consequence of the non-harmonized procedures, breakpoints defined by the French ''Comité de l'Antibiogramme de la Société Française de Microbiologie'' (CA-SFM) [4], the ''Swedish Reference Group for Antibiotics'' (SRGA) [5], the ''Norwegian Working Group on Antibiotics'' (NWGA) [6], the ''British Society for Antimicrobial Chemotherapy'' (BSAC) [7], the ''Commissie Richtlijnen Gevoeligheidsbepalingen'' (CRG) from the Netherlands [8], and the German ''Deutsches Institut für Normung e.V.'' (DIN) [9] vary over a broad range. Therefore, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) has approached this subject. The standing committee of the ESCMID – the European Committee on Antimicrobial Susceptibility Testing (EUCAST) – has achieved a harmonization of existing methods for MIC testing and procedures of breakpoint definitions, and now co-ordinates the process for setting breakpoints for new antimicrobials. In 2005, the European Medicines Agency (EMEA), the pharmaceutical industry, and EUCAST have agreed on a standard operating procedure which allows EUCAST a formal role in the registration process for new agents. EUCAST has agreed on a model for harmonizing breakpoints for existing antimicrobial agents in Europe. For the aminoglycosides, fluoroquinolones, oxazolidinones, cephalosporins, carbapenems, penicillins, macrolides, and aztreonams, there are now European breakpoints (table available on the website at http://www.eucast.org), and the process is continuing with other agents, and should be completed during 2009 [10, 11].

The different approaches of defining a breakpoint in the past, at present, and perhaps in the future have been summarized recently [12–18]. These authors also describe the impact of pharmacodynamics on breakpoint selection for susceptibility testing. In this article, we do not intend to discuss the different methods to establish a breakpoint, but we want to discuss different philosophies of deriving a breakpoint. Beta-lactams are used as representative examples, since breakpoints for this drug class have been set by using different methods, which will be discussed below.

The Need for a Clear Terminology

Epidemiological Cut-Off Values

The approach to categorize a bacterium as susceptible or resistant is based on the distribution of MIC values of a wild-type population of microorganisms without acquired or mutational resistance mechanisms to a given drug and a non-wild-type population harboring such resistance mechanisms. Ideally, the susceptible and resistant populations are clearly distinct from each other; the drug concentration separating these two populations from each other was originally defined as the ''breakpoint'' [1]. Nowadays, the EUCAST defines this concentration as the epidemiological cut-off value in order to avoid confusion with clinical breakpoints. The EUCAST uses the epidemiological cut-off values as follows: ''The epidemiological cut-off values should be used as the most sensitive measure of resistance development – for measuring resistance development in hospitals and the community, for measuring the effect of interventions and for developing strategies to counteract further resistance development'' [15]. Thus, the epidemiological cut-off value is an early warning system to be used by the microbiologist to detect subtle changes in the susceptibility patterns of pathogens. As the epidemiological cut-off value is: (1) determined exclusively by the distribution of MIC values, and (2) is independent of dosing regimens, it is of value only in detecting the development of microbiological resistance to antimicrobial agents. Therefore, epidemiological cut-off values are almost always considerably lower than the clinical breakpoints. In case a bacterial species may be naturally resistant to a given drug, its epidemiological cut-off value is higher than the clinical breakpoint.

Clinical Breakpoints

However, MIC determinations have been used since the 1960s to guide therapy in a patient. This attempt is fundamentally different from the approach to detect resistance. The pathogen was defined as ''susceptible'' if the

drug concentration in serum was higher than the MIC of the pathogen, and it was deduced, therefrom, that the antibacterial therapy should be efficacious. Consequently, the terms ''susceptible'' and ''resistant'' are used in an entirely different way as originally defined by *Ericsson* and *Sherris* [1]. These terms are now being used to classify a pathogen as treatable or non-treatable, in contrast to the original breakpoint definition aiming at a differentiation between the microbiologically susceptible and resistant sub-populations. Likewise, the original definition of the term ''breakpoint'' is now being used entirely differently in order to predict clinical outcome.

This change in definitions has considerable consequences on the type of information provided. Originally, the breakpoint was a quantitative parameter, i.e., an MIC was reported. The actual classifications into ''susceptible'' or ''resistant'' are strictly qualitative criteria.

As the ''breakpoint'' as formerly defined and used by Ericsson and Sherris [1] is, nowadays, named by the EUCAST as the ''epidemiological cut-off value'' [15], the term ''breakpoint'' has to be redefined as well. The EUCAST committee uses the term ''breakpoint'' by adding the important word ''clinical'' as follows:

> ''The clinical breakpoint should be used in everyday clinical laboratory work to advise on therapy in the patient'' [15].

Pharmacokinetic/Pharmacodynamic Breakpoints

The ''clinical breakpoint'' can be derived by two different approaches: the deterministic and the probabilistic approaches. The principal differences between these approaches will be explained in greater detail below. Basically, both approaches attempt to correlate the MIC of the pathogen and the pharmacokinetics of the antibacterial agent with clinical and/or microbiological outcome. The difference between the two approaches is that the deterministic models use the means of pharmacokinetic parameters and the mode or 90% MIC value; the probabilistic approach factors in the variability of pharmacokinetics in the patient populations to be treated and the variability of susceptibilities within a population of pathogens to be targeted. Breakpoints derived by considering both the pharmacokinetic and microbiological variability and the large number of drug-bug exposures resulting therefrom are defined as ''pharmacokinetic-pharmacodynamic (PK/PD) breakpoints.''

Clearly, PK/PD breakpoints will ''advise on therapy in the patient,'' as defined by EUCAST. Thus, the so called PK/PD breakpoints are clinical breakpoints, too, so that it is ambiguous and inconsequential to term them breakpoint – it is just another approach based on PK and PD parameters and the use of stochastic models to define a clinical breakpoint.

To avoid confusion, the breakpoint terminology should be standardized and identical definitions should be used by the EUCAST, CLSI, and the International Organization for Standardization (ISO). The same holds true for the definitions of susceptibility or resistance criteria which are dependent from the breakpoint definitions.

Approaches to the Evaluation of Clinical Breakpoints

Deterministic approach

Traditionally, the establishment of clinical breakpoints is based on three features of either the antimicrobial agent or the pathogen: (1) distribution of MICs, (2) pharmacokinetics of the antimicrobial agent, and (3) clinical outcome. The approach used by the BSAC to set breakpoints has recently been summarized by *MacGowan* and *Wise* [18]. Other scientific societies use slightly modified criteria, either several formulas based on pharmacokinetics were used (CA-SFM, BSAC, NWGA, and the SRGA [13]), or the mean trough concentration [16] or the average drug level in the middle of the dosing interval [17] were related to the MIC values. Basically, all of these approaches have one aspect in common: the breakpoint is being set by adjusting the mean pharmacokinetic parameters derived from healthy volunteers to the susceptibilities of a population of potential pathogens. Consequently, this approach is deterministic, as variabilities in drug exposures are not taken into account.

One major aspect of the deterministic clinical breakpoint setting is the attempt to not dissect the natural population of wild-type strains. In the past, this procedure was followed by the societies/authorities in the US and in many, but not all, European countries. At present, this approach is one of the principles adopted by EUCAST. The common approach in Europe is to establish one common tentative general clinical breakpoint based on information on the pharmacokinetics and pharmacodynamics of the investigational drug. This information is limited during the early phases of development. As soon as a solid database providing species-specific distributions of MICs is available, it is considered whether adjustments are needed or not. Usually, one common clinical breakpoint for Enterobacteriaceae and *Pseudomonas aeruginosa* will be defined. Figure 1 provides an example of this approach; the MIC distributions of ciprofloxacin (Figure 1A), levofloxacin (Figure 1B), and moxifloxacin (Figure 1C) for *Escherichia coli*, *Klebsiella pneumoniae*, and *P. aeruginosa* are illustrated. The clinical susceptible breakpoint of ciprofloxacin is 0.5 mg/l and that of levofloxacin is 1 mg/l, as defined by EUCAST. For moxifloxacin, a *P. aeruginosa* clinical breakpoint is inappropriate, so the moxifloxacin-susceptible breakpoint for Enterobacteriaceae is 0.5 mg/l; otherwise, the clinical breakpoint would have been 4 mg/l. The epidemiological cut-off

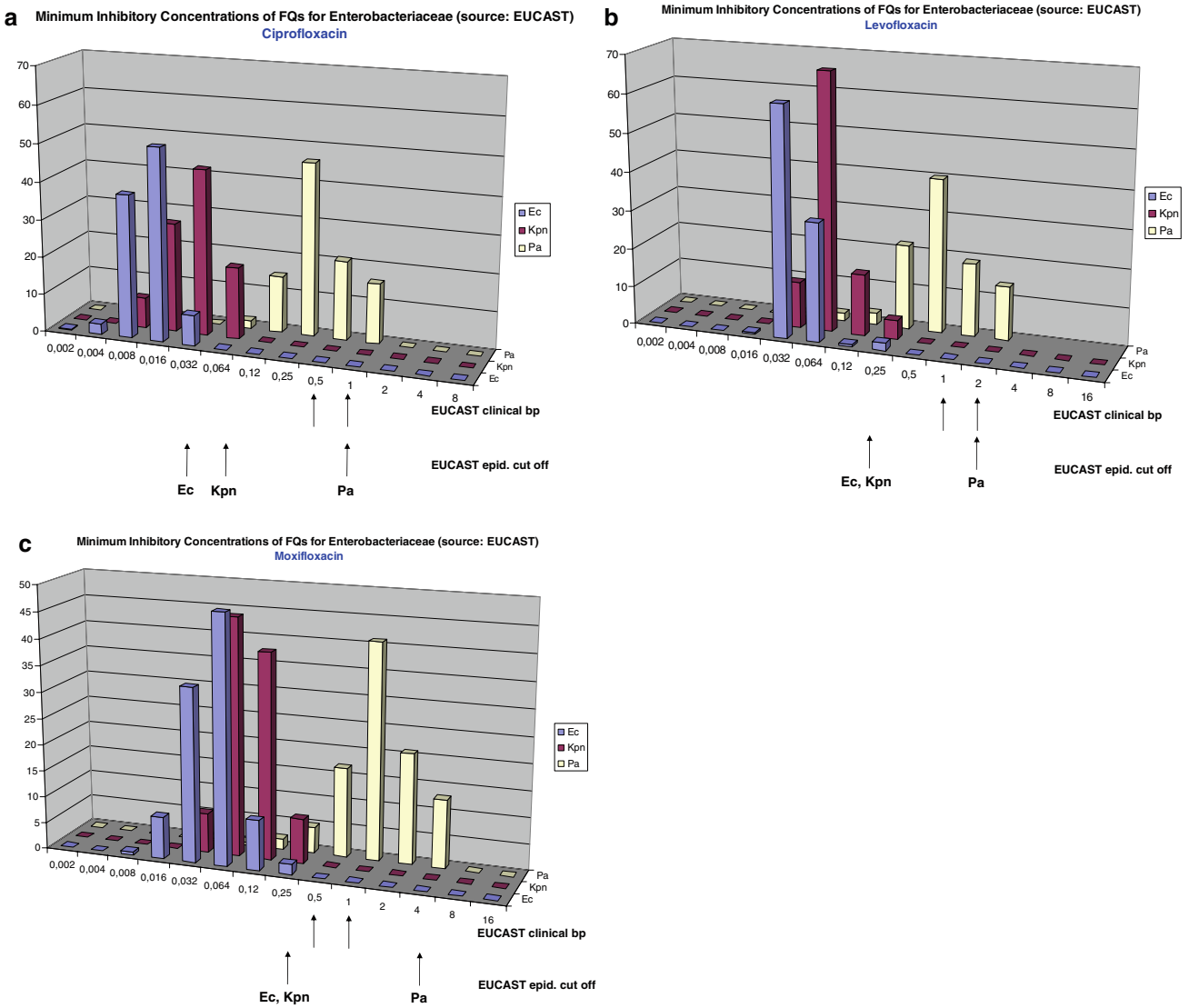

Figure 1. Distribution of aggregated MICs of fluoroquinolones for wild-type *E. coli* (*Ec*), *K. pneumoniae* (*Kpn*), and *P. aeruginosa* (*Pa*). Source: http://www.escmid.org (*clinical bp*, clinical breakpoint; *epid. cut-off*, epidemiological cut-off value). **a**. MICs of ciprofloxacin for *E. coli* (number of observations (no)/number of data sources (ds) (no, 9,124/ds, 16), *K. pneumoniae* (no, 2,653/ds, 6), and *P. aeruginosa* (no, 22,952/ds, 15). **b**. MICs of levofloxacin for *E. coli* (no, 7,514/ds, 4), *K. pneumoniae* (no, 3,258/ds, 5), and *P. aeruginosa* (no, 19,046/ds, 10). **c**. MICs of moxifloxacin for *E. coli* (no, 954/ds, 5), *K. pneumoniae* (no, 3,841/ds, 6), and *P. aeruginosa* (no, 5,536/ds, 7).

values range from 0.032 to 0.5 mg/l for ciprofloxacin, from 0.25 to 2 mg/l for levofloxacin, and from 0.25 to 4 mg/l for moxifloxacin for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Thus, the more active an agent is, the lower are its epidemiological cut-off values and the clinical breakpoints. Although this relation is very obvious for every microbiologist and infectious diseases specialist, the perception of the non-specialized physician is just the opposite: the higher the breakpoint is, the more isolates or species are covered, the more effective the agent in question is. Unfortunately, this message is conveyed by some pharmaceutical companies if deemed appropriate for the support of a given agent. Clearly, such incorrect interpretations should be counterbalanced by the breakpoint setting committees.

Why may the Deterministic Approach not Mirror Clinical Reality?

Previously, clinical breakpoints were established prior to the emergence of accepted knowledge on pharmacokinetics and pharmacodynamics. Since the formulas for deterministic definitions of clinical breakpoints were first proposed, considerable more information became available on the pharmacodynamic characteristics of antibacterial agents [19, 20]. Nowadays, pharmacokinetic and quantitative measures of antimicrobial susceptibilities

have been integrated using pharmacokinetic-pharmacodynamic (PK/PD) models to forecast clinical and microbiological outcomes in the treatment of bacterial infections. Such models utilize patient population pharmacokinetics, susceptibility distributions of the causative pathogen, and PK/PD targets derived from non-clinical models of infection, or clinical data, if available [19–21]. Multiple data from preclinical infection models and recent human clinical trials provide strong evidence that the PK/PD index for cephalosporins against *Streptococcus pneumoniae* that correlates with clinical outcome is the duration of time that drug serum concentrations exceed the MIC of the microorganism (T > MIC); antibacterial effects were observed when free-drug concentrations in serum were above the MIC for approximately 40% of the dosing interval. This PK/PD surrogate index should be attained with a probability of at least 90% (which is arbitrary), although an attainment rate of 95% would be ideal. Simple pharmacokinetic models can be used to calculate the impact of MIC changes on PK/PD parameters [20].

Such calculations show that none of the cephalosporins mentioned in table 1 achieves the target of 40% T > MIC with a probability of 90% for the entire *S. pneumoniae* population. The probability of attaining 40% T > MIC for oral cephalosporins is VERY low; cefuroxime would be useful to reliably treat penicillin-susceptible pneumococci only; cefaclor and cefixime do not even achieve the target of 40% T > MIC for the penicillin-susceptible *S. pneumoniae* [22] (Table 1). Furthermore, maximal cefaclor serum concentrations of 9–16 mg/l are achievable following a 500-mg dose, which are halved in the presence of food. Thus, the resistant-breakpoint of 8 mg/l according to DIN is achievable for a very limited period of time only; in addition, the serum half-life is short (0.5–0.9 h), so that serum concentrations decline rapidly. Consequently, the 24-h exposure of a *S. pneumoniae* strain with a cefaclor MIC of 1 mg/l to this agent is low (T > MIC = 4.1–7.2 h) as compared to a target of 9.6 h. A two-fold change in cefaclor MICs from 1.0 to 2.0 mg/l reduces the PK/PD parameter T > MIC from 4.1–7.2 h to 3.0–4.6 h, which is, at best, less than 50% of the desired target of 40% T > MIC. This low target attainment rate may likely have contributed to the emergence of resistance over the past 25 years.

These examples demonstrate that those clinical breakpoints as defined by the traditional deterministic approaches tend to be too high as primed, or borderline susceptible bacteria may be considered as being fully susceptible. But, as already mentioned, most of the clinical breakpoints have been established prior to the emergence of accepted knowledge on pharmacokinetics-pharmacodynamics.

Why may the Deterministic Approach Foster Resistance Development?

Another aspect which has not yet been considered by any theory, formula, or pragmatic approach to define clinical breakpoints can be clearly illustrated by using again the cefaclor and cefixime examples: the feature not yet addressed is the emergence of resistance to a particular agent and, thus, most probably to the entire drug class amongst pathogens with MICs which are very close to the breakpoint.

The analysis of drug exposures (using FDA-approved pediatric doses) of *S. pneumoniae* isolated from pediatric patients to various beta-lactams is shown in table 2. An appropriate drug exposure is even more important in this patient population, as the emergence of penicillin resistance is related to previous antibiotic therapy. Drug exposures were considered to be appropriate if the corresponding serum concentrations exceeded the MICs of the causative pathogens for 35%–50% of the dosing interval. The target attainment rates (T > MIC = 35%–50%) were calculated for the drugs and doses shown in table 2. Based on these calculations, only the high-dose amoxicillin and ceftriaxone demonstrated overall probabilities of 95% and almost 90%, respectively. However, the cephalosporins failed to attain the target; although there was great variability, the overall probability of achieving the PK/PD target index was low – in particular for cefaclor and ceftibuten [23] (Table 2).

In addition, the emergence of penicillin resistance coincided with the successful launch of less active oral

Table 1
Pharmacodynamic analysis of in vitro susceptibilities of *Streptococcus pneumoniae* (n = 4,489) to cephalosporins (modified from *Mason* et al. [23]).

	Geometric mean MIC (mg/l)			Percent target attainment (40% time above MIC in the dosing interval)		
	Pen s	Pen i	Pen r	Pen s	Pen i	Pen r
Cefaclor	1.04	8.62	60.1	40	0	0
Cefuroxime	0.13	0.68	5.15	100	64	0
Cefpodoxime	0.13	0.45	3.18	94	63	0
Cefixime	0.30	2.89	25.5	69	0	0

The percentage of the dosing interval during which the drug plasma concentration was above the geometric mean MIC was obtained from the plasma concentration vs time curves provided in the package insert

Table 2
Dosing regimens of beta-lactams used for the treatment of *S. pneumoniae* infections and their overall probabilities of achieving a pharmacokinetic-pharmacodynamic (PK/PD) target of 35%–50% T > MIC (modified from [23]) (Regimens represent FDA-approved pediatric doses. The generally accepted PK/PD target for beta-lactams is T > MIC in plasma of 35%–50%. The mean drug concentrations in plasma at this point in time (target) were calculated from published PK data obtained in pediatric patients with otitis media. fu, fraction of unbound drug. The target attainment rate represents the probability in percent of achieving the PK/PD target T > MIC of 35%–50% based on the entire MIC distribution. Number of strains, 977).

Agent	Regimens	Mean drug concentration at target	Target attainment	
			fu (%)	Rate (%)
Amoxicillin	45 mg/kg per day; in three doses	2.7	80	83
Amoxicillin	90 mg/kg per day; in twp doses	8.1	80	95
Cefaclor	40 mg/kg per day; in two doses	0.5	75	5.8
Cefixime	8 mg/kg per day; one dose	0.8	33	18
Cefpodoxime	10 mg/kg per day; in two doses	0.5	72	32
Cefprozil	30 mg/kg per day; in two doses	0.25	60	61
Ceftibuten	9 mg/kg per day; one dose	2.9	35	2.8
Ceftriaxone	50 mg/kg per day; one dose	19.0	10	88

cephalosporins, such as cefaclor and then cefixime [24, 25]. These oral cephalosporins are characterized by a low affinity to the essential penicillin-binding proteins as compared to the anti-pneumococcal penicillins, which have a high target affinity [26]. Oral cephalosporins like cefaclor and cefixime are more potent selectors of penicillin-resistant pneumococci than penicillin and aminopenicillins [25, 27]. The clinical use of cefaclor and cefixime is more often associated with a high rate of resistance development and lower eradication rates than the use of amoxicillin and co-amoxiclavulanate [28–30]. Thus, the shift in the type of antimicrobials within one drug class used clinically is an important contribution to the problem of emerging resistance [31]. Double-tympanocentesis studies in infants and young children with acute otitis media due to penicillin intermediately susceptible and penicillin-resistant *S. pneumoniae* revealed that a cefaclor treatment of 40 mg/kg per day divided into three doses was sub-optimal and resulted in 21% and 62% persistence of the pathogen [32]. This should be considered if the propensities for resistance development, which are different for various agents even of one drug class, should be factored into future breakpoint definitions.

Another example is the emergence of organisms producing extended-spectrum beta-lactamases (ESBLs), which coincided with the popularization of cephalosporins in the 1980s. ESBLs hydrolyze oxyimino-cephalosporins like ceftazidime and ceftriaxone. According to the CLSI (formerly NCCLS) definitions, the cefepime- and ceftriaxone-susceptible breakpoint is 8 mg/l; the resistance breakpoint is 64 mg/l. These breakpoints correspond by ± one titration step to most of the European breakpoints [4, 5, 9], except the BSAC breakpoint [7]. By applying the CLSI breakpoints, most of the ESBL-producing Enterobacteriaceae are susceptible to these cephalosporins. However, if the percent target attainment (T > MIC = 50%) is calculated by using a simplified one-compartment pharmacokinetic model, almost 100% of the bacteria with MICs less or equal to 2 mg/l were adequately exposed [20, 33]. If the MICs are shifted by one dilution step, the likelihood of target attainment falls below 60% and was only 1% at an MIC of 8 mg/l, which corresponds to the CLSI susceptibility breakpoint. For comparison, the probabilities for cefepime target attainment rates for T > MIC of 40% and 50% were 100% each for a 2-g b.i.d. cefepime dose, and ranged from 99% to 95% for a 1-g b.i.d. cefepime dose against an ESBL-producing population of *E. coli* and *K. pneumoniae* [34].

Clinical data from a variety of sources suggest that the probabilities of clinical failures in the use of cephalosporins in treating enterobacterial infections increases in parallel to an increase in the MICs of the infecting organism [33, 35]. The failure rate was 19% in the treatment of patients infected with bacteria with MICs of ≤ 1 mg/l, increased to 33% and 37% for the treatment of pathogens with MICs of 2 mg/l and 4 mg/l, respectively, and amounted to 89% for the treatment of organisms with MICs of 8 mg/l [33, 35].

Therefore, the EUCAST – but not yet the CLSI – has most recently lowered the clinical breakpoints (s and r) for ceftriaxone and cefotaxime to 1 and 2 mg/l, and for ceftazidime and cefepime to 1 and 8 mg/l, respectively (http://www.eucast.org). But still, the EUCAST acknowledges the fact, that despite lowered clinical breakpoints, ESBL-producing bacteria may be missed and that specific ESBL screening tests should be applied. This provision is quite understandable and may mirror clinical reality, i.e., clinical failures, based on the clinical experience quoted above [33, 34].

The attempt to correlate MICs to clinical outcomes clearly underlines the effort of the microbiologist to predict the probability of clinical outcome and underlines the expectation of the clinician to obtain therapeutically relevant guidance from the microbiologist.

These two positions are mirrored by the actual EUCAST definition of the clinical breakpoint:

> ''The clinical breakpoint should be used in everyday clinical laboratory work to advise on therapy in the patient. A microorganism is defined either as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success or as resistant if the activity is associated with a high likelihood of therapeutic failure'' [15].

This definition, by using the term ''likelihood,'' implies that the EUCAST breakpoint calculations are and will, in part, be based on a totally different approach than the ones traditionally used. On the one hand, EUCAST bases breakpoint definitions on pharmacodynamic calculations as well as on the attempts not to dissect the natural population of susceptible wild-type strains.

This new approach involves incorporating the variability in drug exposures observed in a population of patients and the variability of susceptibilities in a population of causative pathogens into a stochastic model [20, 36–38].

Probabilistic Approach

Ideally, pharmacodynamic calculations should include all possible drug exposures for standard-dosage regimens (optimally by using population PK models) and all MIC values likely to be found for the clinical isolates (optimally weighted to disease caused by the pathogen). Therefore, it would be more informative to publish the MIC distribution pattern for a large number of microorganisms, as done by EUCAST on their website [39]. Similarly, serum concentration vs time curves and, consequently, maximal serum concentrations (Cmax) and areas under the serum-concentration curve (AUC) values demonstrate a Gaussian distribution of the individual values.

One concept (but not yet the probabilistic one) was developed by *Schentag* et al. [40–42], who, more than 20 years ago, suggested the concept of dual individualization, which integrates patient-specific data on pharmacokinetics (AUC) and susceptibility of the causative pathogen (MIC) into the dosage optimization specifically for that individual patient. The AUC/MIC ratio as used by *Schentag* et al. [40–42] describes the probability of clinical and microbiological response to ciprofloxacin, tobramycin, and cefmenoxime therapies, respectively.

Additional surrogate parameters like the Cmax/MIC ratio are applicable for those agents and drug classes whose activity is concentration-dependent (e.g., aminoglycosides and fluoroquinolones) and the time of exposure of a bacterium to serum concentration exceeding the MIC (T > MIC; e.g., beta-lactams).

Antimicrobial agents can be categorized by the best fit between these composite parameters (i.e., AUC/MIC, Cmax/MIC, T > MIC) and their efficacy [43, 44]. It has become popular to ''calculate'' these parameters and to base inter-drug class comparisons and judgments of their relative activities and clinical usefulness on these surrogates. The parameters used for these calculations are almost always the means. Furthermore, mean Cmax and mean AUC values are usually obtained from healthy volunteers [45], and most of the MIC values are derived from studies on non-representative or even biased strain collections [46, 47]. Usually, protein binding is not considered, although the free-drug fraction only is antibacterially active and freely diffusible.

Therefore, such calculations based on mean pharmacokinetic and pharmacodynamic values generate single-point AUC/MIC, Cmax/MIC, and T > MIC estimates which provide information on what is possible, but not on what is probable. A practical aspect argues against the use of single-point estimates. Changes in the MIC in particular have a significant impact on PK/PD surrogates: (1), a variability of MICs by ± one titration step, i.e., +100% and –50%, is considered to be still within the normal range; (2) depending on the data source, MICs may vary considerably. For an AUC/MIC or a Cmax/MIC ratio, an increase in MIC has a direct and inverse effect on the ratio, whereas the parameter T > MIC is not proportionally affected by MIC changes. Thus, the choice of an appropriate or convenient or representative MIC value can greatly influence the conclusions drawn from pharmacodynamic analyses based on single-point estimates.

As pharmacokinetic and pharmacodynamic parameters provide a Gaussian distribution, the possible combinations of kinetic and dynamic values are extremely complex. Monte Carlo simulation uses a probability density function to generate random AUC or Cmax and MIC values. Each set of values is a single-point estimate. Once the probability density function for both the pharmacokinetic and microbiological variables are determined, a computer software package is used to simulate a large number of MIC drug exposure scenarios. For each scenario, a single, random MIC value, and a single, random PK value, each corresponding to their individual probabilities, is calculated for that pair. Each set of random values effectively simulates one possible outcome. During the simulation process, a large number of PK/PD outcomes are computed, representing, in the end, a probability distribution. The use of Monte Carlo simulations to integrate PK and susceptibility data is described in detail by *Dudley* and *Ambrose* [20] and *Ambrose* and *Grasela* [36]. Through this methodology, thousands of single-point estimates can be made and their probability plotted. The resultant AUC/MIC or Cmax:MIC probability distributions can be utilized to examine the entire range of possible AUC/MIC and Cmax:MIC ratios and the probability of achieving each of them [11, 21, 48].

Consequently, this method to calculate clinical breakpoints can be defined as the probabilistic approach,

since almost all probable drug-bug exposure rates are integrated into this model.

One rather simple but clinically very important consequence of the use of the probabilistic approach is that the entire range of probable drug exposures – from low to high – is modeled, as opposed to the deterministic approach, which is not governed by drug exposure but by the distribution of MICs in relation to mean drug concentrations. Consequently, breakpoints as defined by the probabilistic approach tend to be lower than the ones defined deterministically.

Why Consider Population-Specific Pharmacokinetic Characteristics?

Another important aspect which should be considered in the context of clinical breakpoint definitions is the difference of pharmacokinetics in healthy volunteers and in the infected patients. In the landmark study on the pharmacodynamics of levofloxacin published by *Preston* et al. [49], evidence was presented first and foremost that the prospective use of PK/PD modeling identified an optimal dose that ensured clinical efficacy and predicted a sub-optimal regimen which will select resistant sub-populations of pathogens. Second, the correlation of clinical outcome probabilities with PK/PD indices revealed that the site of infection modulates the target attainment rate. Third, this study clearly demonstrated that pharmacokinetics in infected patients [49] and in young healthy volunteers [50] are not alike and are much more variable in patients than in volunteers. Surgical patients and patients in the intensive care unit (ICU) will likely have different PK characteristics than healthy volunteers [51–54]. Age and gender, organ function, and the underlying disease may affect the pharmacokinetics of many drugs (summary in [55]). Therefore, it is likely that the probabilities for clinical success or the PK/PD target attainment rates will be different in certain patient groups as compared to healthy volunteers. Using ceftazidime as an example [56], it was demonstrated that probabilistically derived clinical breakpoints were ≤ 4 mg/l in healthy volunteers and 0.5 mg/l in patients with cystic fibrosis or patients in the ICU [56]. This example demonstrates that a data set (the absolute values as well as their distribution) derived from a small population of healthy volunteers is not always valid for certain patient populations. It would be ideal, therefore, to use population-based approaches differentiating between defined groups of infected patients and healthy volunteers to calculate PK/PD indices. Clearly, information about pharmacokinetics in patients is not available during the early phases of drug development. But as the clinical trials proceed, and at the latest when clinical breakpoints will be established, this important information has to be considered and should be factored into the procedures for breakpoint definitions.

In addition to its usefulness in breakpoint definitions, the use of PK/PD parameters may likely be of relevance to reduce the likelihood of emerging resistance [57]. Retrospective analyses of published data generated by adopting preclinical infection models supported this hypothesis [33, 34, 46, 58, 59]. Not the expression of a resistance phenotype as such predicted poor outcome but rather, a strain harboring a mutation towards resistance will have an increased MIC relative to its wild-type counterpart. This elevated MIC value is often close to but not beyond the susceptibility breakpoint as defined deterministically (see above). Consequently, therapeutic efficacy will be maintained provided that the mutant strain will be exposed to almost identical PK/PD surrogates as the wild-type isolate. However, these subtle increases in MICs pass unnoticed in routine susceptibility test procedures because of too high (deterministically defined) breakpoints, so that the exposure of such mutants is inadequate; this, in turn, fosters further resistance development.

In a most recent comprehensive review of PK/PD studies conducted in both experimental animals and humans, convincing evidence was presented that PK/PD measures and probabilistically derived breakpoints predicted drug efficacy and minimized the amplification of drug-resistant sub-populations [21]. Data like these strongly support the introduction of probabilistically defined clinical breakpoints in order to ensure adequate exposures of the pathogens to the antibacterial agents and to reduce the likelihood that resistant sub-populations may be selected due to inadequate drug exposures.

Conclusion

It has been a long journey since the first attempts in 1971 to define *microbiological* breakpoints to the actual approach to define *clinical* breakpoints. Clearly, this implies that none of the various approaches is right or wrong, and that the different approaches reflect different philosophies and mirror the tremendous progress made in the understanding of pharmacodynamic properties of different classes of antimicrobials. Previously, the clinical breakpoints have been set deterministically. More recently, and in parallel to an increasing knowledge in the pharmacodynamics of antibacterial agents, probabilistic models have been applied to derive clinical breakpoints on the basis of pharmacokinetic and microbiological variability. Probabilistically derived clinical breakpoints are lower than the deterministically established clinical breakpoints. Thus, not only the methods used to derive the breakpoints are different, but the breakpoints themselves are different, too. Consequently, all of the breakpoints for a given drug class have to be defined by using the same method; otherwise, the recommendations derived from ''mixed breakpoints'' would be unbalanced. The use of the probabilistic approach may likely have a great impact on treatment regimens and, thus, on clinical and microbiological outcomes, and – at least as equally important in an era of

continuously increasing drug resistance – may likely reduce the selection of resistance.

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