

Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections

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The distinction between bactericidal and bacteriostatic agents appears to be clear according to the *in vitro* definition, but this only applies under strict laboratory conditions and is inconsistent for a particular agent against all bacteria. The distinction is more arbitrary when agents are categorized in clinical situations. The supposed superiority of bactericidal agents over bacteriostatic agents is of little relevance when treating the vast majority of infections with gram-positive bacteria, particularly in patients with uncomplicated infections and noncompromised immune systems. Bacteriostatic agents (e.g., chloramphenicol, clindamycin, and linezolid) have been effectively used for treatment of endocarditis, meningitis, and osteomyelitis—indications that are often considered to require bactericidal activity. Although bacteriostatic/bactericidal data may provide valuable information on the potential action of antibacterial agents *in vitro*, it is necessary to combine this information with pharmacokinetic and pharmacodynamic data to provide more meaningful prediction of efficacy *in vivo*. The ultimate guide to treatment of any infection must be clinical outcome.

Antibacterial therapy, a keystone in modern medical practice, provides one of the only pharmacologic treatments that cure disease. Many clinicians have accepted certain assumptions and generalizations concerning antibacterial therapy that are not necessarily based on rigorous scientific evidence. One of these hypotheses is that agents with *in vitro* bactericidal activity are preferred to agents with *in vitro* bacteriostatic activity.

Newly discovered antibacterial agents are tested *in vitro* not only for ability to inhibit the bacteria, but also to determine whether the new agent actually “killed” the bacteria. Although it would seem preferable

for an antibiotic to kill the offending bacteria rather than to merely inhibit it, the clinical importance of an *in vitro* bactericidal action being better than a bacteriostatic action has rarely been documented.

Because of resistance to currently available antimicrobial agents used to treat infections with gram-positive bacteria [1–7], current approaches to therapy for these infections must be reappraised. Ultimately, the treatment target should be achievement of a good clinical outcome (clinical/bacteriologic cure and no relapse) with the least toxicity. This review addresses the relevance of *in vitro* bacteriostatic versus bactericidal action as it relates to the clinical efficacy of antibacterial agents to treat gram-positive bacterial infections.

DEFINITION OF BACTERIOSTATIC/ BACTERICIDAL ACTIVITY

The definitions of “bacteriostatic” and “bactericidal” appear to be straightforward: “bacteriostatic” means that the agent prevents the growth of bacteria (i.e., it keeps them in the stationary phase of growth), and

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“bactericidal” means that it kills bacteria. In reality, there are *not* 2 pure categories of antimicrobial agents (one that exclusively kills bacteria and another that only inhibits growth). Rather, those agents that are called “bactericidal” usually fail to kill every organism (if, for instance, the inoculum is large) within 18–24 h after the test, and most so-called “bacteriostatic” agents kill some bacteria within the 18–24 h after the test—often more than 90%–99% of the inoculum, but not enough (>99.9%) to be called “bactericidal.” The *in vitro* microbiological determination of whether an antibacterial agent is bactericidal or bacteriostatic may be influenced by growth conditions, bacterial density, test duration, and extent of reduction in bacterial numbers. The clinical definition is even more arbitrary. Most antibacterials are better described as potentially being both bactericidal and bacteriostatic.

Microbiological definition. Various *in vitro* microbiological techniques to determine the bactericidal activity of antibacterial agents against different isolates include the minimum bactericidal concentration (MBC), time-kill curve, and serum bactericidal titer (SBT). Each technique may provide useful information, but the clinical values of these techniques are limited by technical problems and difficulty in practical interpretation [8–10]. Until recently, techniques had not been standardized for determination of the MBC, and they remain unstandardized for SBT determination [11]. The techniques to determine MBC have varied considerably over time and between laboratories, therefore providing only a snapshot in time and place for a particular organism. Reproducibility of test results remains an ongoing problem in the inter- and intralaboratory standardization of such tests. Thus, the definition of bacteriostatic or bactericidal activity for an antibacterial agent applies only to the particular organism (or even strain) against which it has been tested under the particular test conditions used.

Quantitative susceptibility testing is usually performed by making 2-fold dilutions of the test antibacterial agent in a liquid culture medium, inoculating it with a standard number of microorganisms, and incubating it at 35°C–37°C for ~18–24 h. The amount of antibacterial that inhibits visible growth (inhibitory phase) of the microorganism is called the “MIC.” Subcultures of samples obtained from clear tubes or wells (in the case of microtiter testing) are made on a medium (usually solid) free of antibacterial agents and reincubated for an additional 18–24 h to determine the MBC, which is the lowest concentration of an antibacterial agent that either totally prevents growth or results in a $\geq 99.9\%$ decrease in the initial inoculum (i.e., a 3- \log_{10} reduction in colony-forming units [cfu]/mL) on subculture.

Guidelines for performing bactericidal tests were published in 1999 by the NCCLS [12]. Critical methodology components for MBC include an inoculum of $\geq 5 \times 10^5$ cfu/mL and a subculture volume of 0.1 mL to accurately predict whether $\geq 99.9\%$

of the bacteria were killed. Although a $\geq 99.9\%$ reduction in viable bacterial density in an 18–24-h period is the generally accepted definition of bactericidal, there is no evidence that a somewhat more or less stringent number might not be equally useful in predicting clinical utility. It is also unclear why a cutoff incubation time of 18–24 h was chosen in this test, although it was probably so it would be the same as the standard cutoff time for MIC susceptibility testing of nonfastidious bacteria. Perhaps extension of the incubation time from 18–24 h to 36 h or even 48 h would change the classification of many antibacterial agents from bacteriostatic to bactericidal, or vice versa. Similar speculation regarding the size of inoculum and growth characteristics of the culture can be made. In summary, MBC values represent the result of an *in vitro* test in which the fixed, static concentration of an antibacterial agent is being tested against an initially fixed concentration of bacteria in an aqueous medium. This does not correspond with the *in vivo* situation, in which antibacterial and bacterial concentration in various body fluids and tissues may fluctuate widely [13, 14].

Bacteriostatic activity has been defined as a ratio of MBC to MIC of >4 , but numerous technical problems and other factors can affect determination of the ratio [8, 15–18]. Some of these factors may have an important impact on the interpretation of the *in vivo* situation. Stationary-phase cultures result in diminished killing rates [19] to such an extent that the bactericidal effect of some cell wall-active antibacterial agents can be eliminated (e.g., against nongrowing or slowly growing phases of *Staphylococcus aureus*) [20]. *In vitro* determinations of bactericidal activity are almost invariably performed against logarithmic growth-phase cultures, which may not reflect the *in vivo* growth pattern of bacteria, and this may have clinical implications. MBC test conditions may also affect results [18, 21]; sufficient quantities of antibiotic may be transferred in subcultures to inhibit growth of surviving organisms [22, 23]; and oxygenation, pH [24–26], and incubation duration [21] or temperature [24, 27] can affect reliability. In addition, the osmolarity and ion content of the medium can impact interpretation of the MBC.

Time-kill curves have been used to determine the kinetics of bacterial killing *in vitro* but not routinely to determine whether an antibacterial agent is bacteriostatic or bactericidal. They can be useful in distinguishing whether bacterial killing is concentration and/or time dependent: concentration-dependent bacterial killing occurs when the rate and extent of killing increases with progressively higher antibacterial concentrations (e.g., for aminoglycosides and fluoroquinolones), and time-dependent killing occurs when increasing antibacterial concentrations to more than the MIC do not result in proportional increases in killing (e.g., for β -lactams and oxazolidinones). The area under the serum concentrations curve that exceeds the MIC is critical for time-dependent killing.

SBT is the greatest serum dilution that usually kills 99.9% of the initial bacterial inoculum after incubation for 18–24 h. The clinical utility of SBT has not been proven, and SBT has not been routinely adopted in clinical practice to monitor or direct individual patient care.

Two related phenomena that interfere with bacterial killing are paradoxical effect and tolerance. Eagle and Mussleman [28] reported that a high proportion of gram-positive organisms showed a “paradoxical” effect such that, when the concentration of penicillin was increased to more than the optimal bactericidal concentration, the bacteria died at a reduced rather than an increased rate, so that the maximal effect was obtained only within a relatively narrow zone [15, 17, 29–36]. In vitro phenotypic “tolerance” has been defined as an MBC that is ≥ 32 times the MIC. Animal studies have indicated that penicillin-tolerant streptococcal endocarditis is more difficult to cure than that due to nontolerant strains [35, 37]. Therapeutic failure has been documented in humans with penicillin-tolerant *S. aureus* pneumonia [38] and increased morbidity/treatment failure in endocarditis [39–41].

Clinical definition. Bacteriostatic and bactericidal categorizations in clinical practice are not absolute and can lead to false assumptions concerning antibacterial therapy, especially if other major antibacterial pharmacokinetic/pharmacodynamic parameters are ignored. It is important to distinguish microbiological and clinical definitions. Evidence is scarce to support MBC testing for individual patient care [8], even though it is an accepted in vitro parameter in evaluating a new antibacterial agent. Antibacterial agents with the lowest MICs or MBCs may not be preferable to an agent with a higher MIC or MBC—for example, good in vitro bactericidal aminoglycoside activity against *Salmonella enterica* serotype Typhi does not translate to clinical efficacy.

Exceptions to the clinical definition. Some broad classes of antibacterial agents considered bacteriostatic can exhibit bactericidal activity against some bacteria on the basis of in vitro determination of MBC/MIC values. At high concentrations, bacteriostatic agents are often bactericidal against some susceptible organisms [33]. Macrolides are considered to be one of the classic bacteriostatic drug classes, but erythromycin, azithromycin, and clarithromycin have shown bactericidal activity in vitro against *Streptococcus pyogenes* and *Streptococcus pneumoniae* [42–45]. Similarly, chloramphenicol is bactericidal against *S. pneumoniae* but bacteriostatic against *S. aureus* and group B streptococci [46–49]. Clindamycin may be bactericidal in vitro, depending on the organism and growth conditions [50, 51]. In vitro, linezolid has bacteriostatic activity against staphylococci and enterococci but bactericidal activity against streptococci, including *S. pneumoniae* [52, 53].

Similarly, antibacterial agents that are considered to be bactericidal as a broad class may only exhibit bacteriostatic activity

in vitro. At low concentrations, bactericidal drugs may merely exhibit bacteriostatic activity. Quinupristin-dalfopristin is generally considered to be bactericidal in vitro against most strains of staphylococci and streptococci but is bacteriostatic against *Enterococcus faecium* [54, 55]. Although all quinolones are bactericidal, they have a single concentration at which they are most bactericidal: the paradoxical effect of decreased killing at higher concentration most likely results from dose-dependent inhibition of RNA synthesis [56, 57]. Furthermore, the robustness of the bactericidal activity of a drug depends on bacterial load and growth phase. For in vitro determination of bactericidal activity, the bacterial cell density is 10^{5-6} cfu/mL of actively growing culture, whereas microbial concentrations can be as dense as 10^{8-10} cfu/g of infected tissue [58]. These dense populations are predominantly nongrowing bacteria. Organisms present at high loads are therefore slower growing than those used for in vitro MBC measurement [13] or represent bacterial populations that are predominantly in a nongrowth phase [58]. The lack of efficacy with a high bacterial load has been demonstrated in vivo for various bactericidal antibacterials. These include vancomycin and cefotaxime in experimental endocarditis due to gram-positive bacteria [59, 60] and penicillin (but not clindamycin) in experimental mouse thigh infection with *Clostridium difficile* and *S. pyogenes* [61, 62].

FACTORS AFFECTING CLINICAL OUTCOME OF ANTIBACTERIAL THERAPY

Nonmicrobiological factors affect response to therapy, including host defense mechanisms, site of infection, underlying disease [13, 14, 63], and an antibacterial agent’s critical intrinsic pharmacokinetic and pharmacodynamic properties.

Inadequate penetration of the infection site is one of the principal factors related to failure of antibacterial therapy. The active drug needs to reach the bacteria in appropriate body fluids and tissues at concentrations necessary to kill or suppress the pathogen’s growth.

The ability of antibacterial agents to cross the blood-brain barrier is an important consideration for the treatment of meningitis. Aminoglycosides do not efficiently penetrate bronchial secretions [64]; therefore, pulmonary infections require higher doses of the drug [65]. Availability of free (active) drug is affected by the degree of protein binding. Higher doses than those reflected by in vitro data may be necessary clinically. To treat intracellular bacteria, efficient penetration of cells is necessary (e.g., macrolides typically concentrate in phagocytes). By enveloping themselves in a fibrous exopolysaccharide glycocalyx, bacteria are protected from host defenses and the action of antibacterials [66–69]: clindamycin may impact bacterial eradication by direct antibacterial activity as well as by inhibiting the development of glycocalyx biofilms by *S. aureus* [70,

71] and adherence via fibronectin binding of *S. aureus* to host cells [72].

CLINICAL SITUATIONS IN WHICH BACTERICIDAL ACTION IS CONSIDERED NECESSARY

Endocarditis. Bacteria within cardiac vegetations may reach very high concentrations (10^8 – 10^{10} organisms per gram of tissue). At such densities, rates of metabolism and cell division appear to be reduced, resulting in a reduced susceptibility to bactericidal effects of cell wall–active agents. The bacteria are dormant, being surrounded by fibrin, platelets, and possibly calcified material [73, 74]. Bacteria considered susceptible to various antibacterials in most situations are relatively resistant in endocarditis [75]. Clinical cure is often achieved, but prolonged administration of relatively high doses of a bactericidal cell wall–active antibacterial agent is generally required for true sterilization of the vegetation to kill any dormant bacteria when they start to produce cell walls with division.

Recognition of the potential importance of bactericidal activity for treatment of endocarditis dates back to early observations [76], but it is significant that a bacteriostatic agent (clindamycin) has been used with success in the treatment of staphylococcal endocarditis (20 [74%] of 27 cases were cured) [77]. This is similar to later studies in which 21 (70%) of 30 patients with *S. aureus* endocarditis were cured with nafcillin, with or without gentamicin [78], and 11 (73%) of 15 were cured with penicillin, methicillin, or nafcillin [79]. Enterococcal endocarditis represents a particular dilemma, with pathogens often showing resistance to penicillins, aminoglycosides, and vancomycin, the agents primarily considered in the treatment of endocarditis due to gram-positive organisms [4]. As single agents, they exhibit bacteriostatic activity against susceptible enterococci in vitro [80]. The combination of penicillin or vancomycin with gentamicin or streptomycin is required for therapy. Linezolid, which is bacteriostatic in vitro against enterococcal species, has cured some cases of vancomycin-resistant *E. faecium* endocarditis [81,82].

Meningitis. It is often considered that antibacterials for the treatment of meningitis need to be bactericidal not just because of the need to eradicate infection as rapidly as possible but also because of the poor immunologic competence of the CNS. However, penetration of many antibacterials into CSF is poor or variably dependent on the degree of inflammation. Certain antibacterial agents that are generally considered to be bacteriostatic—tetracycline [83], chloramphenicol [84], linezolid [85, 86], and trimethoprim-sulfamethoxazole [87, 88]—penetrate CSF efficiently and have been used successfully to treat gram-positive bacterial meningitis. However, in animal experiments, ampicillin has been more effective than chloramphenicol in *S. pneumoniae* meningitis [89], and clinical outcome has been poor in pediatric patients with penicillin-resistant *S. pneumoniae* meningitis treated with chloramphenicol [90]. Rare cases of vancomycin-resistant *E. faecium* meningitis have been successfully treated with linezolid [85, 86].

Osteomyelitis. Because drug penetration may be poor in osteomyelitis because of decreased vascular supply, it might seem logical to choose a bactericidal agent for therapy; however, clindamycin, a bacteriostatic agent, achieves high concentrations in bone and is considered an appropriate agent for the treatment of gram-positive bacterial osteomyelitis [91, 92]. Successful outcome of osteomyelitis is determined by adequate surgical debridement and choice of an antimicrobial agent to which the organism is susceptible, rather than that agent's bactericidal properties.

Neutropenia. The use of bactericidal antibacterial therapy has been suggested to treat bacterial infections in severely neutropenic patients [93, 94]. Supporting evidence appears to rely more on presumed synergistic activity of combination therapy, usually a β -lactam plus an aminoglycoside. Gram-positive bacteria have now become an important difficult-to-treat cause of infection in neutropenic patients [93, 95, 96]. Bacteriostatic agents have not been adequately studied in these patients.

Table 1 lists bactericidal and alternative bacteriostatic antibacterial classes used for serious gram-positive bacterial infections.

Table 1. Bactericidal versus bacteriostatic antibacterial classes for serious gram-positive bacterial infections.

Condition	Traditional bactericidal agent class	Alternative bacteriostatic agent class
Endocarditis	β -Lactam or glycopeptide, with or without an aminoglycoside	Lincosamide, ^a oxazolidinone ^b
Meningitis	β -Lactam, glycopeptide	Chloramphenicol, lincosamide, ^a oxazolidinone, ^b tetracycline, trimethoprim-sulfamethoxazole
Osteomyelitis	β -Lactam, glycopeptide	Lincosamide ^a
Neutropenia	β -Lactam, with or without an aminoglycoside	None

^a Clindamycin.

^b Linezolid.

DISADVANTAGES OF BACTERICIDAL ACTION

Some data indicate that potentially adverse clinical consequences may result from the rapid lytic action of bactericidal antibacterial agents [97, 98]. Endotoxin surge is well documented after antibacterial therapy in the CSF of infants with gram-negative bacterial meningitis [99, 100]. In meningitis due to *S. pneumoniae*, rapid death of microorganisms results in the production of increased cell wall fragments and intracellular pneumolysin, which intensify the WBC response and prostaglandin release, resulting in increased cerebral edema and the high mortality rate for pneumococcal meningitis [90, 101]. Even chloramphenicol is lytic to *S. pneumoniae*, so no matter what agent is used, a marked inflammatory reaction may occur as a result of bacterial lysis.

ADVANTAGES OF BACTERIOSTATIC ACTION

Exotoxins of staphylococci and streptococci may produce toxic shock syndrome. Although these bacteria are usually susceptible to clindamycin, its bacteriostatic action had for some time been considered a disadvantage, and bactericidal antibacterial agents were preferred. However, clindamycin has been shown to completely inhibit toxic shock syndrome toxin-1 production by *S. aureus* in both growth- and stationary-phase cultures [102]. At high bacterial loads, clindamycin is also more effective than penicillin in reducing mortality of experimental thigh infection with either *Clostridium perfringens* [62] or *S. pyogenes* [61]. Clindamycin is now considered a major component of therapy for staphylococcal and streptococcal toxic shock syndrome [103]. Bacteriostatic agents inhibit protein synthesis in resting slow-growing bacteria not affected by bactericidal β -lactams.

CONCLUSIONS

The greater the ignorance, the greater the dogmatism.

Attributed to William Osler, 1902

The presumption of the superiority of in vitro bactericidal over bacteriostatic action in the treatment of gram-positive bacterial infections is intuitive rather than based on rigorous scientific research. The distinction between the terms "bactericidal" and "bacteriostatic" might appear to be clear according to in vitro definition, but this only applies under strict laboratory conditions, is inconsistent for a particular agent against all bacteria, and is considerably more indistinct clinically. Most authors agree that the possible superiority of bactericidal activity over bacteriostatic antibacterials is of little clinical relevance in the treatment of the great majority of gram-positive bacterial infections. The one proven indication for bactericidal activity is in enterococcal endocarditis. Meningitis is usually treated with

bactericidal agents, but bacteriostatic agents, such as chloramphenicol and linezolid, have been used effectively. In vitro bacteriostatic/bactericidal data may provide information on the potential action of antibacterial agents, but this is only one of many factors necessary to predict a favorable clinical outcome.

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