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*Research Article*

# Evaluating Bacterial-Antimicrobial Agent Interactions In The Laboratory To Understand The Challenge of Resistance

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## A B S T R A C T

In this overview, multiple laboratory interactions of antimicrobial agents and bacteria used to define resistance are described. The ability to demonstrate antimicrobial resistance through standard and innovative laboratory techniques is characterized Using innovative techniques in the microbiology laboratory. Potential resistance to bacteriophage therapy, previously thought to be an alternative approach to resistance, may be shown to not be the panacea once considered.

**Keywords:** Antimicrobial inhibition; Heteroresistance; Lethality; Resistance; Tolerance

## 1. Introduction

Amid the concerns of increasing antimicrobial resistance and associated deaths[1], is the need to identify and characterize antimicrobial inhibition, resistance, heteroresistance and lethality as interpreted from testing in the clinical microbiology laboratory. Further complicating clinical interpretations are that bacteria in states of persistence and latency are unresponsive to antimicrobial action. The generally accepted metrics to determine response or resistance to therapy are measurements of inhibition, the minimum inhibitory concentration (MIC) and of lethality, the minimum bactericidal concentration (MBC). These metrics have been well studied and accepted as necessary endpoints to evaluate clinical response or resistance in the laboratory[2].

In the following discussion, characteristics and implications of these endpoints in identifying and minimizing the impact of antimicrobial resistance are reviewed.

## 2. Methods Review and Implications

The earliest method for assessing antimicrobial susceptibility was demonstrated by Fleming following his serendipitous observation of the activity of *Penicillium notatum*[3]. Clearly this was the genesis of the agar diffusion technique for determining antimicrobial susceptibility that is in place today in many clinical microbiology laboratories. Fleming's initial observations of antimicrobial activity included growth inhibition of five bacterial genera: Staphylococcus, Streptococcus, Pneumococcus, Gonococcus and Diphtheria. He also noted the absence of any activity against two other species: *Escherichia coli* (*B. Coli*) and *Haemophilous influenzae* (*B. influenzae*)[3]. There were only two endpoints detected: no growth or growth.

From that original demonstration and observation evolved the agar disk diffusion assay and then the tube dilution assay in which antimicrobial agents were diluted in the growth medium. The tube dilution assay yielded the endpoint of the MIC - the antibiotic concentration at which bacterial turbidity could not be observed. The MIC intersects with the breakpoint concentration of the antimicrobial agent and its clinical interpretation by the laboratory as either "susceptible" (S) or "resistant" (R) or "intermediate" (I). Subculturing apparently clear tubes (broth

determined to be at the MIC) in the dilution assay beyond the MIC results in determining the MBC[2]. The categorical values of "S," and "R," and "I" with numerical equivalents are typically defined by two major consensus groups, the Clinical Laboratory Standards Institute (CLSI) in the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in the European Union. Automated antimicrobial testing systems (ASTs) gained widespread use in clinical laboratories in recent years but no AST has ever achieved universal acceptance by CLSI or EUCAST for determining the MBC.

The transition from bacterial inhibition (the MIC) to killing (the MBC) can be profound and reliance solely on the MIC frequently results in clinical treatment failure. What is the reason for this failure? Apparently bacterial populations, although arising from a single colony on agar medium, are not always identical. An apparent single bacterial population that appears to be susceptible as determined by the MIC, may include a sub-population of bacteria that are resistant to the same antimicrobial agent. Bacteria that exhibit this duality of susceptibility are referred to as heteroresistant. These bacteria can pose a challenge for practitioners to diagnose and treat serious infections.

Analogous to the expression of antibiotic heteroresistance is the capability of some bacterial species to become tolerant to antimicrobial action. Unlike the MIC or MBC endpoint, there is no universal metric that clearly defines tolerance. This leads to the misclassification of "tolerant" strains as "resistant." Tolerance is defined as the ability of bacteria to survive when treated with antibiotics, while resistance is the ability of bacteria to grow even when exposed to antimicrobial therapy. Research has aimed to define a measure for this observation, the quantitative indicator of tolerance, the MDK, "minimum duration for killing"[4]. It appears that there are extant specified bacterial genes associated with increased tolerance.

Laboratory studies that can further aid the clinician in understanding the patient's response to therapy are the drug levels which measure the actual serum concentration of the antimicrobial agent after administration to an individual. Additionally, the serum cidal concentration, which evaluates the potential microbial killing ability of the drug directly from patient's serum, can be determined for a given bacterial-antimicrobial pair. These techniques, in addition to the MIC and MBC, are particularly beneficial in the management of complex infections.

At antibiotic concentrations below the MIC, bacterial growth may be inhibited and controlled by phenomena known as the post-antibiotic effect (PAE). This effect can be observed following drug removal. Typically, antibiotics that kill bacteria by interfering with protein synthesis, eg, aminoglycosides, macrolides, chloramphenicol and tetracyclines or the quinolones that inhibit DNA replication, exhibit this effect. The PAE provides information for adjusting dosage regimens of antimicrobial agents when managing serious infections[5].

In addition to the conventional AST systems there are several molecular platforms to identify resistance susceptibility in bacteria. These platforms rely on the polymerase chain reaction (PCR) or Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry. The PCR systems can detect the bacterial gene responsible for resistance; MALDI-TOF systems detect the protein moiety resistance component. In either technology, the potential for resistance to a specific antibiotic can be detected, but this information does not forecast the actual expression of resistance[6].

Another novel technology that can simultaneously identify the bacterium (genus and species) and determine which antimicrobial agents to which it would be susceptible or resistant, is next- generation sequencing (NGS)[7]. [**Figure 1** summarizes the laboratory techniques reviewed]. The technology is capable of determining the order of nucleotides in an entire genome. It can thus identify a bacterium, characterize novel pathogens, as well as determine potential susceptibility or resistance genes that confer response to antimicrobial agents. As indicated earlier and similar to MALDI-TOF, the detection of genomic susceptibility/ resistance genes does not necessarily represent expression of that potential.

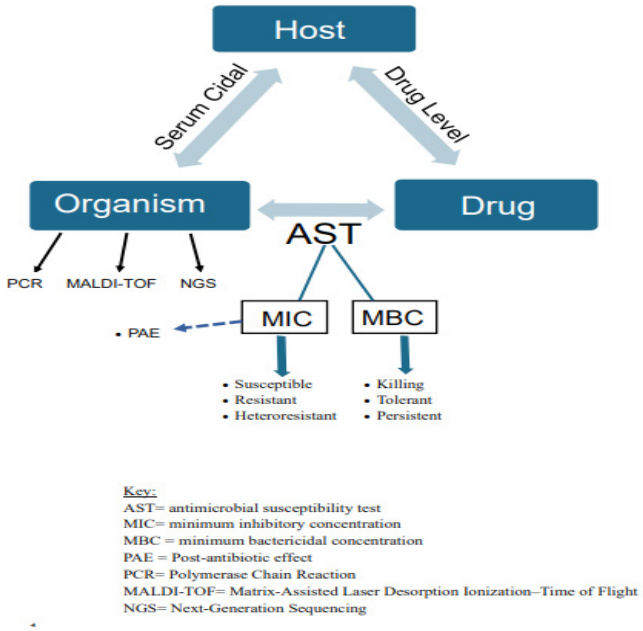


**Figure 1:** Outline of the triad of host, drug and organism and the varied tests to evaluate their interactions.

## 3. Future Considerations

Are all cells in a bacterial population identical? Evidently, as indicated previously, they are not. Therein lies the varied and sometimes unpredictable response of bacterial populations to antimicrobial agents. But what is as yet unknown is whether all such bacteria would be susceptible to bacteriophage therapy, a novel approach to antimicrobial resistance[8].

Although this approach would seem to be a unique solution to thwart resistance, some bacteria, specifically *Klebsiella pneumonia*, have been shown to overcome infectivity and killing by phage by producing anti-phage proteins[9]. Can PCR, NGS or MALDI-TOF measure genes or proteins in phage to help us understand and predict bacteriophage resistance? This is not yet known. Clearly, more work in this area is needed to understand and prevent anti-phage responses by bacteria to solve the global problem of resistance.

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## 5. Conflict of interest

The author declares no conflicts of interest in the publication of this paper.

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