THE EVOLUTION OF ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS

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INTRODUCTION

It is reasonable to conclude that antibiotic testing did not precede the concept of antibiotics, therefore, it is logical to start the review with penicillin and the work of Alexander Fleming. It should be noted that the history of antibiotic testing is no different than other topics in the history of science, there are always relevant predecessors to whatever event is chosen as the starting point for the subject. It would be negligent to fail to mention the work of Paul Ehrlich as well as several early observations that seem worthy of note before focusing on the evolution of the events leading to the contemporary field of antimicrobial susceptibility testing.

There is a brief review of the subject by Lechevalier and Solotorobsky. Anyone interested in the early observations that preceded the work of Fleming should consult this 1965 publication. The published works of Pasteur, Koch and especially Paul Ehrlich contain many references to antibiosis. Along with these observations are descriptions of laboratory methodologies that support the concept of antibiosis. This methodology essentially consisted of measuring activity by employing: (i) test tubes containing broth (but technically not a MIC determination), (ii) a loss of motility, or (iii) animal protection studies. Although these observations were significant, they did not establish practical methodologies that were predictive of clinical outcome with the possible exception of animal studies conducted by Ehrlich. Since this review focuses on in-vitro testing, the importance of the work mentioned above is dutifully noted. However, before leaving this subject, recognition should be given to two early events that illustrate the types of laboratory observations that were made in the early years following the establishment of bacteriology as a separate branch of science. In 1874, William Roberts observed that liquid medium in which the mold Penicillium glaucum was growing, could not be easily contaminated with bacteria. In 1876, John Tyndall
as noted by Lechevalier and Solotorobsky made a similar observation that broth supported the growth of either bacteria or mold, but rarely both. These examples illustrate that in the early years of bacteriology, laboratory workers were observing the effects of inhibiting substances as well as inhibition between organisms. It also illustrates that broth was employed to establish the inhibiting effect of these metabolic products on the growth of bacteria in the laboratory. These and other observations were essentially isolated events that did not lead to the establishment of an organized series of continuing studies.

It is almost an understatement to note that Paul Ehrlich was the first bacteriologist to devote considerable effort focused on discovering therapeutic cures for infectious diseases employing chemical agents. As director of the Königliches Institute, he stated that the task of this new institute was to find "specific chemotherapy of infectious diseases" by employing products generated in the laboratory rather than by use of substances found in the serum of humans or animals. His pioneering work in chemotherapy established this subject as a separate field of scientific inquiry, however, his techniques tended to be quite organism-specific, and relied heavily on in-vivo animal studies. It was 20 years later that work on penicillin began the series of events that the modern day concept of in-vitro testing became established. This chain of events formed the basis of modern antimicrobial susceptibility testing methodology. This work was initiated by Alexander Fleming and his contemporaries in the 1920's.

Alexander Fleming made his initial observation on the inhibitory effect of what eventually became known as penicillin on solid media by observing an area of growth inhibition of staphylococcal colonies adjacent to a Penicillium contaminated agar plate. For purposes of the current review, this event will be taken as the starting point for the evolution of contemporary susceptibility testing methodologies.

It is important to state the sources of information for the next three sections of this review. A search of the literature for authoritative surveys on the evolution of antimicrobial susceptibility testing revealed a great variety of works by many authors. Most of these reviews are organized by methodology rather than in a confluent chronological order. The present authors felt that for analysis purposes, a better sense of how the contemporary field of susceptibility testing developed could be obtained by creation of a master chronology that could then be divided into time periods with certain landmark events chosen to represent particular periods of time. By employing this method, a series of short analyses could be made for each of the time blocks.

To accomplish this goal, five major works were selected that, in the judgment of the authors, demonstrated particularly sound and well-researched material. These five works were used to create a chronological table of significant events in the history of the subject. The chronology is presented at the end of this review. The following are listed in the chronology: The date the method was published, the reference source for each method, the primary investigator or author(s) of the paper describing the method, and a brief description of the methodology. This chronology begins in 1924 and continues through 1988. In the text that follows, reference is only made to the investigator and date. If one is interested in more information concerning any of the methods described in the text, the chronology can be consulted with the corresponding reference source. It should be noted that only certain events were selected from the chronology for inclusion in the text. Although this may not be a conventional reference method, the authors believe that this system contributes to the flow of