SENSITIVITY TESTING OF ANTIBIOTICS: A BRIEF REVIEW

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ABSTRACT

In today’s scenario microbial species are not susceptible with conventional naturally derived or chemically synthesized antibiotics. This increase in the antimicrobial resistance has lead to more chances of illness and higher death rates following treatment failures and greater health care costs. The cause behind this resistance is repeated overlapping of microbial receptor with a particular antibiotic whereby microbes tend to alter their own binding mechanism and exhibit less susceptibility with that particular antibiotic. Ultimately antibiotics lose their sensitivity towards various types of microbes and are not able to show a desired response. Thus, it becomes merely crucial to investigate the efficiency of an antibiotic for a particular type or species of micro-organism. This efficiency of antibiotics can be evaluated through various sensitivity tests viz: Dilution method, disk diffusion method, E-test etc. which provides the investigator with an opportunity to assure himself whether a particular antibiotic has a promising effect or not against a given microbe.

Keywords: - antibiotics, dilution, disk diffusion, efficiency, microbes, sensitivity

How to cite this article: Jindal K, Goswami M (2020): Sensitivity testing of antibiotics: A brief review, Ann Trop Med & Public Health; 23(S15): SP231536; DOI: http://doi.org/10.36295/ASRO.2020.231536

1.0 INTRODUCTION

1.1 Basics of antibiotics

Antibiotic is a word which is obtained from Greek word i.e. anti means against, biotic means life of microbe. Antibiotics are chemicals which are derived from micro-organism (i.e. bacteria and fungi) and have capability to kill or slow down the growth of pathogenic micro-organism. Collectively all the antimicrobial drugs are called antibiotics and are classified as antibacterial, antifungal, antiviral and anti-parasitic.

Now a days, antibiotics are among the most frequently prescribed medicines. Some antibiotics possess the property to kill the pathogenic microbes and are called as “cidals” while some are capable of preventing the growth of microbes and are called as “statics”. But in general, antibiotics hinder the cellular function of microbes whereby microbes are unable to synthesize their own cell wall (Cell wall is very crucial component of bacteria for their reproduction).

Though antibiotics are used to save lives from various microbial infections but like other drug products these also tend to show some side effects. However, such effects can make life miserable, after a prolonged use but are not fatal.
1.2 Classification of antibiotics

Antibiotics can be classified on the basis of their following pharmacological attributes:

(i) Mode of spectrum
(ii) Effect on microbe
(iii) Mechanism of action

**Mode of spectrum:** A variety of antibiotics have been used to cure a broad range of infections. Such antibiotics are called as broad-spectrum antibiotics i.e. they are effective against both Gram(+) ve and Gram(-) ve bacterial infections e.g. Penicillin, Cephalosporins, Tetracycline etc.

While some antibiotics are limited to very few species of microbes and hence are called as narrow-spectrum antibiotics. eg. Bacitracin (it is effective against Gram (+) ve) and Polimixins(these are effective against Gram (-) ve only).

**Effect on microbe:** Antibiotics exhibit their effect on microbial growth by two ways either by killing them, i.e. bactericidal action e.g. Rifampicin, Aminoglycosides, and Cephalosporins etc. or by preventing their growth, i.e. bacteriostatic action e.g. Chloramphenicol, Trimethoprim, and Tetracycline etc.

**Mechanism of action:** Antibiotics generally act on any of the three portions of a particular micro-organism:-

(i) Cell wall (made up of NAM and NAG which are responsible to form glycopeptidic linkage and this linkage is further involved in synthesizing the cell wall of a microbe). Antibiotics suppress the effect of microbe by inhibiting the synthesis of this cell wall e.g. Beta-lactams and Bacitracin

(ii) Ribosomal chain, this chain is responsible for synthesizing a number of proteins by micro-organisms and these proteins are pathogenic for other living organisms. This is where the antibiotics show their effect by inhibiting the synthesis of such proteins.

(iii) Most of the antimicrobial agents undergo binding to either of the subunits ofribosomal strand of bacterial RNA and thus hinder their protein synthesis. e.g. Macrolides, Aminoglycosides, Chloramphenicol and Tetracycline or by targeting their nucleic acid i.e. DNA and RNA

Whereas some antibiotics act by targeting the initial components responsible for the synthesis of bacterial RNA or DNA, leading to hindrance in their normal cellular processes and this ultimately affects probability of multiplication of bacteria and thus the chances of their survival. Examples: Rifampin,Quinolones, Metronidazole,

1.3. Mechanism of antibiotic resistance

In today’s scenario, Antibiotics have made possible the treatment of many clinical conditions resulting from bacterial infections. These antibiotics turned out to be so effective that we are bound to use them and consider their effectiveness for granted. However, in certain cases we may even regret their random and prolonged use. For example: inappropriate dosing,duration of treatment, indiscriminate prescribing and even the availability of antibiotics to the general public without any prescription. Such a dependency ultimately leads to an increase in the development of resistance to antibiotics among various common human pathogens. This ultimately threatens the significant development of antibiotics.¹

Antibiotics are one of the most crucial components of any medicine, but this is our hard luck that bacterial agents have capability to resist these antibiotics. Antibiotic-resistant bacteria are the organisms which cannot be killed by
using such antibiotics. When bacterial agents are exposed to a given antibiotic many times, their occur changes in
the bacterial configuration which ultimately makes them resistant to a particular antibiotic².

There are a number of possible ways through which micro-organisms are capable of showing antibiotic resistance. For instance, they have a tendency of altering their cellular structure using their internal mechanism thus, rendering a given antibiotic to be ineffective. In certain cases, such bacterial agents tend to inactivate or neutralize the antibiotic. Moreover, bacteria can make use of their tendency to transfer the genes coding for a particular antibiotic resistance between themselves, whereby making it possible for that bacteria to acquire resistance against the same even if it has never been exposed to that antibiotic.

Further, there are even the worsened cases of antibiotic resistance:
   a) where these antibiotics have been used to cure the ailments in which they render negligible efficacy (e.g. viral infections),
   b) or the cases rendering the prophylactic use of such antibiotics rather than in the treatment. Though certain cases can be cured with more powerful medicinal agents, but still there are few infections that cannot be cured even with the newer developing drugs.

1.4 Aim of carrying out susceptibility tests

The main purpose of any antibacterial susceptibility test is to predict the least amount of an antibacterial agent (existing or experimental) capable of inhibiting the visible growth of the bacteria being investigated. Owing to the serious and rapidly growing problem of antibiotic resistance, there is an urgent need to develop the techniques of resolving such issues. Here forth are given the most commonly employed methods to test antibiotic sensitivity.

2.0 ANTIBIOTIC SENSITIVITY TESTING METHODS

2.1. DILUTION METHODS

This method involves dilution of broth medium followed by various different concentrations of antimicrobial agent. Micro-dilution tests are carried out in a microtiter format³ using about 50-100 microlitre of total volume of broth. Macrodilution tests are carried out in standard test tubes using broth about 1.0 ml broth volume.

In both of the micro and macro dilution methods, a Minimal Inhibitory Concentration or MIC is recorded. This, MIC is the minimum concentration or the least amount of the antibiotic that is capable of preventing the growth of the concerned microbial species. But this method is valid only, if the positive control shows growth and the negative control shows no growth.

Another such method is agar dilution. This method is similarly based on the principle of calculating the least amount of the antibiotic which has been diluted in a specific series and is capable of inhibiting bacterial growth⁴.
Fig 1.1 showing the dilution method

A particular bacterial isolate can be tested for resistance to twelve different antibiotics using this agar plate. Any clear zone around a particular disc is the zone of inhibition indicating the probability of a particular isolate to exist in the presence of the antibiotic being tested. In fig 1.1, a large zone of inhibition is indicated by the yellow coloured disk; whereas the discs with no zone of inhibition reveal the resistance exhibited by the concerned species.

But one should keep in mind that susceptibility of the antibiotic cannot be directly interpreted on the basis of presence of zone of inhibition; there is a need to measure the zone width and then compare it against a reference standard which further provide us with a range of measurements further categorized as susceptible, intermittently susceptible or resistant.

Fig 1.2 showing the measurement of zone of inhibition (E.coli)

For example, fig 1.2 depicts that this particular E. coli isolate is resistant to Ampicillin exhibiting an inhibition zone of 10.1mm around Ampicillin.

Annals of Tropical Medicine & Public Health  http://doi.org/10.36295/ASRO.2020.231536
2.2. DISK DIFFUSION METHOD

The disk diffusion susceptibility method\(^5\)_\(^6\) is quite simple, well-standardized and easily feasible technique. Owing to its efficacy, ease and cost effectiveness, this procedure has turned out to be the frequently applied technique for detecting any sort of antimicrobial resistance in clinical conditions\(^7\).

Disc diffusion method\(^8\) is generally carried out using Mueller-Hinton agar medium. This procedure makes use of commercially prepared disks already saturated with a standardised amount of concerned antibiotic, followed by their even dispersion and are then placed onto the agar surface with little pressure. Then occurs an immediate outward diffusion of the test antibiotic through the discs, leading to a concentration gradient of antibiotic in the medium in a manner that more amount of antibiotic can be seen near the discs with least amount far apart from the discs. Further, after an overnight period of incubation, each disc is observed for any sort of bacterial growth. Now, if one observes that there is negligible growth in the concerned disc, this indicates that the particular strain is inhibited by a given antibiotic.

The zone of inhibition is thus referred to the area surrounding disc containing antibiotic and rendering zero or negligible growth. This gives an idea about the minimum concentration of the antibiotic that is enough to resist the tendency of particular isolate to grow. The inhibition zone is determined in terms of millimetre and is further compared to a standardized data differentiated in three categories. However, on the basis of this simple qualitative test classifying the isolate as susceptible, intermediate or resistant we cannot have an accurate measurement of minimum inhibitory concentration. But still many commercialized devices have capability to determine an approximate value of minimum inhibitory concentration in certain cases\(^9\)_\(^10\).

Many attempts have been made till today to improve the efficiency of this method, including betterment of the basic procedure and interpretation of zone sizes\(^11\). Also, it involves an idea of using a partially automatic reading technique along with a digitalised camera linked to the lab computers. Besides these limitations steps have been taken to enhance its use globally\(^12\)_\(^13\).

This disk test is widely used to test Influenza strain, Neisserial strain for meningitis and streptococciby employing special media, suitable environmental conditions, and standard interpretation chart.

2.3. E-TEST

E-test is a widely employed test which makes use of a strip made of plastic saturated with a gradually decreasing concentration of a given antibiotic\(^14\). There is a numerical scale on the strip corresponding to the concentration of the antibiotic contained therein\(^15\). This technique has turned out to be more effective for quantitative determination of resistance to antibiotic by the concerned bacterial strain. However, this test is comparatively costlier as it involves the use of a separate strip for every given antibiotic.

A profound correlation has been observed between the E-test results and the MICs obtained using broth or agar dilution methods\(^14\)_\(^16\). Despite this, some systematic differences still exist towards slower or higher minimum inhibitory concentrations calculated by the E-test against certain organism-antimicrobial agent combinations\(^2\)_\(^17\). These sometimes represent a potential shortcoming of the same\(^17\).
2.4. AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY TESTING SYSTEMS

With a view in mind to minimize the technicality issues and reduce the time involved in any kind of preparation, a variety of devices have been designed and commercialized to enhance the availability of such microdilution panels that can be easily prepared along with the automated instruments.

Most of the automated systems provide us with possible automatic inoculation, automatic ability to read and interpret\(^1\). Such automated devices render the features of being convenient and faster (in certain cases results can be obtained within hours), besides the limitation of the higher cost involved in the purchasing, processing and maintaining of the machinery. Examples include: Walk-Away device (Dade International, Sacramento, Calif.), Micronaut (Merlin, Bornheim-Hesel, Germany), Vitrek device (bioMerieux, France), Phoenix (BD Biosciences, Maryland) etc.

2.5. MECHANISM SPECIFIC TESTS

Based on the principle of directly detecting the presence of a particular resistance mechanism, some methods have been developed to determine the resistance. For example, used to detect the enzyme (i.e. Chloramphenicol acetyltransferase) involved in the metabolism of Chloramphenicol with colorimetry and estimation of \(\beta\) lactamase can be carried out using a chromogenic cephalosporinase test.

2.6. GENOTYPIC METHODS

In some cases, there is a possibility of finding the specific genes rendering antibiotic resistance by making use of the fact that such resistance traits are genetically encoded. Further, this resistance depends on the dominancy of genes eleven. So it becomes necessary to keep in mind that the presence of such a gene is not merely responsible for any kind of failure during treatment. Despite all, these DNA-RNA based detections methods are significantly more efficient and faster. Two such techniques are as follow:-

2.6.1. Polymerase chain reaction (PCR): It is a widely employed technique used to isolate the specific DNA sequences to a certain extent. In this process, the sample DNA undergoes several cycles of denaturation followed by conjugation of particular primers to the targeted sequence, further extending this sequence resulting in repetition of a duplicate DNA sequence, as facilitated by a thermostable polymerase exponentially, to a level that can be detected visually using fluorescence techniques.

2.6.2. DNA hybridization: It follows the fact that pyrimidines specifically pair up with purines. Hence, a genetically sequenced probe can conjugate with uncoiled or distorted DNA from the given sample, until or unless their sequences are complementary in nature.

Keeping in view the basic fundamentals, many attempts have been made to further enhance the specificity and sensitivity of these standardized methods. Examples include the development of deoxy ribose nucleic acid chips and deoxy ribose nucleic acid arrays etc.
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