Editorial on antibiotic susceptibility testing

Microbiology was accepted as a clinical science with the advent of chemotherapeutic agents which changed the world of infections. With the advent of Penicillin, it became imperative to identify the bacteria that caused infection. Microbiology became a sister speciality of pathology and entered the hospital laboratory. Then, it followed a cascade of antibiotic discoveries that made doctors virtual gods waving a wand and wiping away formerly deadly diseases, such as puerperal sepsis, pneumonia, meningitis, syphilis, gonorrhoea and even the lowly urinary tract infection.

Antibiotic use became the order of the day, and every fever was tamed with an antibiotic. In the microbiology laboratories, scientists and technicians toiled at Petri dishes and culture media to accurately predict the antibiotics which would be of use. Many of the early antibiotics such as Penicillin, Tetracycline, Chloramphenicol and Co-trimoxazole had their own target infections where cure was the norm. Hence, susceptibility tests were rarely performed and not much relied upon as they took at least three days and were cumbersome to do.

By the late 1940s, antibiotic resistance emerged first among the staphylococci which became resistant to Penicillin. This led to a need for susceptibility testing. Testing for sensitivity to antibiotics started as a well in an agar plate, then as a disc and later more accurate techniques like broth dilution and agar dilution evolved. Standards for testing and more rapid methods of testing were needed.

According to Fleming, "it was found that the colonies of staphylococci around the mould were transparent and rapidly undergoing lysis. The broth in which the mould was grown had acquired marked inhibitory, bactericidal and bacteriolytic properties."[1] These, in short, are the two methods for sensitivity testing still followed today, disc diffusion and broth or agar dilution using purified forms of chemicals released from moulds. Dr. Fleming, it is interesting to note, worked in the Inoculation Department, St. Mary's Hospital, London. His discovery elevated this to the Microbiology Department in later years. In his paper, he remarks that the new chemical, Penicillin, was totally free from irritant effects of common disinfectants used then and may be considered for use to disinfect wounds by local application. He even shows that it is quite bactericidal on Gram-positive organisms, but not effective on Gram negatives, and hence may be used in media to isolate Gram negatives from a mixture. He has made MIC tables showing the different dilutions at which Penicillin kills all the major Gram positives. These included not only staphylococci but also streptococci, pneumococci and the "green" streptococci, gonococci and Anthrax.

From that state, the antibiotics and the science of microbiology has evolved to reach clinical microbiology where infections are treated based on reports from the microbiology laboratory. Hence, the present-day microbiologist and the present-day laboratory have a great responsibility in the outcome of treatment and also in the prevention of antimicrobial resistance.

Hence, what is the role of the modern microbiology laboratory in treatment of infections caused by bacteria?

1. Determine the organism responsible
2. Sensitivity testing by disc diffusion or find MIC
3. Advice on therapy.

Organism Responsible

The accurate identification of the organism responsible for the infection has gained importance. In the earlier days, reports such as "Coliforms isolated" and "non-fermenter isolated" were common. It was also felt that there was some difference in the *in vitro* susceptibility and the *in vivo* effect of certain antibiotics. Once the exact identification is got, the behaviour of the bacteria becomes clear, for example, *Pseudomonas aeruginosa* is inherently resistant to Tigecycline, Stenotrophomonas is sensitive only to Co-trimoxazole and any other sensitivity seen on the plate is only an *in vitro* effect. Among Gram positives, *Enterococcus gallinarum* is inherently resistant to Vancomycin and certain alpha lytic streptococci are resistant to Penicillin. Identification of yeasts is extremely important. *Candida krusei* and *Candida glabrata* are inherently resistant to Fluconazole.

Sensitivity Testing

Although it may sound straightforward, there are many problems associated with doing, interpreting and reporting sensitivity test results.

Putting up a sensitivity test

The medium used is Mueller-Hinton agar with a particular thickness, pH and salt content. This is available as pre-prepared media and can be reconstituted. The

antibiotics used depend on the identity of the bacteria. However, the identity will also be known only on putting up the identification tests overnight. Based on Gram reaction, colony characteristics and certain rapid tests, the probable identity is known.

Which are the antibiotics to be tested, from a vast array available? Do you need to test every available type of cephalosporin/quinolone? Here is where the Clinical and Laboratory Standards Institute (CLSI) has given us the answer to a certain degree. Every laboratory should prepare a panel of antibiotics for different categories of bacteria, such as Gram positives and Gram negatives broadly, streptococci, enterococci and pneumococci, coliforms and non-fermenters. It is very convenient if you can also write down the discs to be put together in one Petri dish. In case of Gram positives which have a clear distinction between first and second line, they may be put in different plates. However, Clindamycin and Erythromycin should be put in the same plate to look for *D*-test positivity. In case of Gram negatives, instructing the technician to place certain discs close together will help in understanding the mechanism of resistance and inducing the expression of certain kinds of resistance genes such as AmpC. The understanding that many of the antibiotics belong to a group and only one of the groups needs to be tested is very useful in reducing the burden on the technician and time spent in looking at sensitivity plates. There is now a great need to know the sensitivity pattern and using the data for epidemiological purposes such as making an antibiotic policy; hence, all the antibiotics necessarily have to be tested at the same time. The way they should be arranged on the Petri dish is of utmost importance and must be guided by a clinical microbiologist.

If data on number of extended spectrum beta lactamase producing bacteria or those with capability to produce AmpC beta lactamase or metallo beta lactamases are needed, the specific tests will have to be done along with routine testing.

Reading a sensitivity test

This is also a duty of the clinical microbiologist. A disc diffusion test plate has many patterns on it that can be used in giving useful advice on treatment to the clinician. Synergy between two drugs is indicated by a merging of the zones with no indentation. Antagonism on the other hand draws a straight line between discs, because the area where the two drugs merge is where the bacteria grow best. Here, the drugs neutralise each other. This is also why it is important to give instructions on which discs should be put together on a Petri dish. CLSI gives instructions on which are the surrogate antibiotics, for example, Cefoxitin for Oxacillin resistance, Oxacillin for Penicillin resistance and use of Cefoxitin to interpret sensitivity for all beta-lactams including first-generation Cephalosporins. While writing down the sensitivity results, this has to be kept in mind always. For all doubtful results, an MIC may have to be done. The *E*-test has made this an easy exercise and automated systems give the MIC automatically for all antibiotics.

Reporting

It may seem that once the sensitivity results are ready, the report can be automatically generated. It is here that the skill of the clinical microbiologist comes into play once again. Restrictive reporting has its advantages and disadvantages. The advantage is that in hospitals where there is no active antibiotic stewardship programme, the microbiologist can restrict reporting to the first-line drugs, thus preventing the microbiology laboratory from initiating misuse of antibiotics. Many a time, de-escalation which is necessary to preserve high-end antibiotics does not happen because the microbiology laboratory reports all antibiotics for all bacteria. The disadvantage is that sometimes the drugs reported may be toxic to the kidney, and time may be spent in contacting the laboratory and the microbiologist, thus compromising the safety of the patient. Hence, once the microbiologist commits to reporting only certain antibiotics, he should also ensure that he is ready to be contacted at all times for advice on treatment if an alternative is needed.

Routine reporting of MIC or printing out the sensitivity report from the automated sensitivity test instrument can lead to certain problems. MICs can confuse the clinician, unless it is followed up with an advice on the drug of choice and its dose based on the MIC. The printout from the machine will not include facts such as use of surrogate markers. Oxacillin or Cefoxitin sensitivity will not be extrapolated to use of first-generation Cephalosporins, unless reported as such.

Another aspect of reporting is the remarks section at the bottom of the report. This can be used to great advantage, from commenting on the quality of the specimen to giving advice on the exact dose and duration of treatment. The report with comments also assures the clinician that a caring and responsible person is monitoring all the results.

The job of the clinical microbiologist does not end there. A full follow-up of the patient is necessary to note that the course of treatment advised is completed with no adverse reactions or unexpected reversals that need repeat tests.

In this issue of the journal, the special article data have been analysed, keeping in mind all the above-mentioned points. It is seen that most centres have panels of antibiotics for different bacteria, use

first-line antibiotics only, if sensitive and also mention the drug of choice.[3]

Good interactions with clinicians with monitoring of patients regularly are necessary adjuncts to antibiotic sensitivity testing and reporting.

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Access this article online

Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/jacm.jacm_14_17

How to cite this article: Raja K. Editorial on antibiotic susceptibility testing. J Acad Clin Microbiol 2017;19:2-4.