appeared on 13th Nov 2013

Choosing the right drug

Finding the right antibiotic to use may now be possible in minutes, says S.Ananthanarayanan.

The regrouping of bacterial forces against the efficacy of antibiotics is a major challenge before health administrators. The manufacture of antibiotics started in the 1920s, after Penicillin was isolated and since then, antibiotics have changed the face of public health. It is estimated that 3 million lives were saved thanks to Penicillin during World War II and many erstwhile life-threatening conditions are now routinely treated. But the manner in which antibiotics work permits target organisms to evolve to become immune, if given the chance – and this chance is what bacteria get very often while doctors fumble about which antibiotic to use.

Marlene Fredborg, Klaus R. Andersen, Erik Jørgensen, Aida Droce, Tom Olesen, Bent B. Jensen, Flemming S. Rosenvinge, Teis E. Sondergaard1, working in Denmark report in the Journal of Clinical Microbiology that they have developed a technique to drastically reduce the time taken to find the right antibiotic. This could mean near elimination of ineffective drug use, which helps generate drug resistant strains of bacteria.

Antibiotic action

Antibiotics, like the body's antibodies, act by forming an exact fit, at the molecular level, with the surface of an invading cell. This can either prevent the cell from attacking its preferred target, or disrupt reproduction or production of necessary proteins by the cell. At the onset of infection, there is a first phase, when mechanisms in the body survey the intruder and design antibodies, by trial and error. But once the body has overcome a mild infection, the antibody design is there and during a subsequent attack, the body can act very fast and can be said to have become immune.

Certain pathogens, like viruses, routinely undergo changes in their surface features. A descendant strain of the pathogen may hence develop a different profile at the relevant part of its surface and would be able to evade the antibody. This is how we have new strains of flu, for instance. And the body, in such a case, has to develop a new antibody, if it has the time.

Antibiotics are powerful agents against bacterial attack and were first extracted from micro-organisms which could kill bacteria. They were later grown in cultures of these organisms and now, several antibiotics are synthesized in laboratories or factories. But, as non-living molecules, the action of the antibiotic is only by means of a matching fit with a part of the target bacterium's cell surface, to immobilize, sterilize or liquidate the bacterium.

Like in the case of viruses, this shape-specific feature is the means by which a bacterium can escape antibiotic action. In any population of bacteria, while there would be individual differences, there would be some individuals who differ from others in the part that is attacked by the antibiotic. But as the antibiotic would be effective against the bulk of the bacterial colony,

the numbers of the surviving individuals would not be viable and they would also perish. In the case of viruses, they multiply rapidly and evolve, but the body does deal with the new strain as well as possible. In the case of bacteria, the strains do not evolve as fast and known curative antibiotics usually stay effective.

The trouble is when there is insufficient or ineffective use of antibiotics. Then the colony of bacteria survives but the immune members are selectively spared. The result is that the colony that will now grow with a larger proportion of immune individuals. Further ineffective use of antibiotics would lead to more concentration and soon there may be a strain of antibiotic-resistant bacteria. With increasing human population and international trade and travel, such strains, sometimes called '***superbugs***', could become widespread.

Ineffective use

Different antibiotics are effective against different classes of bacteria. Certain antibiotics, called ***broad spectrum***, cover a wide range of bacteria, with a compromise, either in the effectiveness of action or by the extent of side effects, for example, of destroying good or helpful bacteria. At the onset of an infection, the physician would prescribe the appropriate antibiotic, based on the diagnosis. But in cases where the culpable organism is not identified, it may be necessary to carry out tests to identify the organism, or the effective antibiotic.

In such a case, the physician would first prescribe a 'broad spectrum' antibiotic and switch to the specific one after the tests results are in hand. This is usually a smart way to act, except that with growing human population, overcrowding, and communication and, to an extent, advertising by drug companies, there is a lot of such broad spectrum drug prescription going on. The result is that during the period of milder action, at least till the '***drug sensitivity***' results come, there is concentration of any resistant component of the pathogen population. Over repeated instances, the resistant component may become viable on its own and what we end up with is a drug resistant strain, which may need the discovery of a new antibiotic!

Finding the right drug

The method to discover which antibiotic would be effective against an unknown pathogen is to create a culture of the bacteria and to observe how different antibiotics act. In the usual, ***Disc Diffusion Test***, the infecting organism is grown as a 'lawn' on a gelatin base and then paper discs soaked in different antibiotics are placed over the culture. The antibiotic diffuses around each disc in circles, getting dilute as the circle expands. After a few hours, it is seen that the discs are surrounded by circles of poor bacterial growth and the largest circles, known as the boundary of

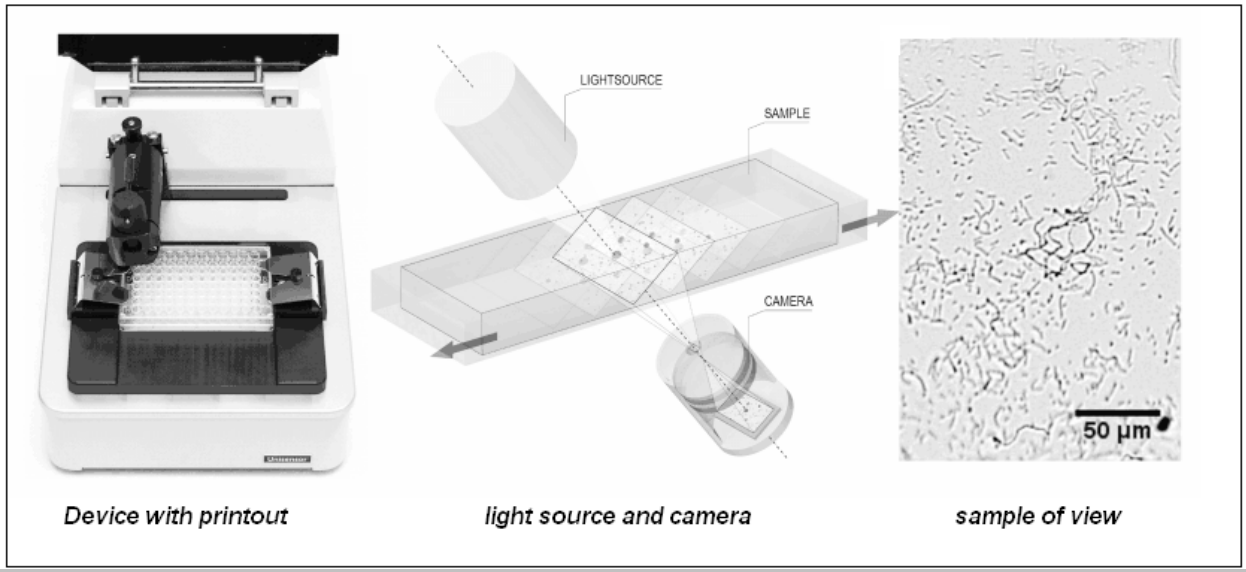
Zone of inhibition - differeint concentrations of drug

minimum inhibitory concentration (MIC), obviously contained the most effective antibiotics. A refined form of this test is with the antibiotic soaked in a strip, to which a calibrated scale is attached. The strip gives rise to an oval of inhibited growth and how far the oval extends along the scale is a measure of the effectiveness of the antibiotic.

The trouble with this kind of testing is that the process takes many hours and the results would usually be available only on the next day, if not later. This period of delay in starting correct treatment, and the milder, wide spectrum treatment in the interim, is seen as promoting antibiotic resistance in bacteria. While there is research on improve diagnosis and public education to prevent self medication, which compounds the problem, the real answer would lie in drastic reduction of the time it takes to assess the sensitivity of the bacteria to different antibiotics.

The development

The Denmark group, supported by Danish firm Unisensor Ltd, has speeded up the pace by watching bacterial growth through a microscope, in real time, in place of waiting for circles to form on a glass plate. The arrangement is an illuminating unit, a lens and a digital camera connected to a computer. The light passes the sample at a shallow angle, to increase the volume studied. The camera has a short focus depth and resolution that is as good as a 200X microscope.



Images are continuously acquired, to form an image stack, with the volume of the oblique portion of sample viewed. For assessing the effectiveness of antibiotics, the growth of *e-coli*, with and without the antibiotic, Polymyxin B, was scanned every minute and processed with a custom algorithm. It was found that statistically significant difference in the rate of growth in this single bacterium culture could be detected in six minutes. In complex cultures, the results could come in 30 minutes.

The time to complete the antibiotic susceptibility tests (AST) could thus be reduced to less than half. This would lead to early diagnosis and early use of the correct antibiotic – to combat the threat of multidrug resistance of bacteria, say the researchers. The method could also be used for a broad range of applications in bacteriology and clinical and veterinarian settings, they say.