Superbugs have evolved into a serious community health problem. Will we be able to conquer the menace that we have ourselves created through the indiscriminate use of antibiotics? Scientists are working hard to counter this challenge.

How We Help Superbugs Thrive?

There are many situations in which bacteria may find an environment suitable for development of resistance. When a person is treated with antibiotics, about 30% is absorbed and the rest passes through the body into the sewage system. Antibacterial soaps and disinfectants used in homes and hospitals are also washed into the sewer. Animal breeders mix antibiotics with farm feed, often indiscriminately, to increase the animal’s weight. Such use not only contaminates the meat but also increases the variety and quantity of antibiotics in the sewage.

Antibiotics are not readily degradable. Ultimately the sewage enters a treatment plant, which encourages the growth of bacteria to digest the sewage. During this process, in the presence of low levels of antibiotics, some bacteria may develop resistance. When the digested sludge is dried and used as manure, some of the farm products may get contaminated with bacteria and enter the food chain. In addition, sewers may directly contaminate the drinking water system, as often happens in our country. Both aid the spread of resistant bacteria in the community.

Thus, in a large population of bacteria there may be a few that have developed resistance to antibiotics. When an infected person is treated with antibiotics, the susceptible ones perish, leaving behind the resistant ones, which will multiply at the opportune moment. Next time when the same antibiotic is given to the patient, it may not be effective in controlling the infection. When a class of bacteria becomes resistant to a particular drug, the pharmacologist develops a new kind of antibiotic. It takes more than a decade to develop an antibiotic and the bacteria become resistant to even the new drug in due course.

The case of Tuberculosis in India illustrates how various shortcomings in the healthcare system have led to the emergence of Extremely Drug Resistant TB (XDR-TB). Poor compliance of drug course by patients, the tendency of doctors to over-prescribe antibiotics, improper screening of patients and spurious or sub-standard drugs floating around in the market are some of the factors that have led to the strengthening of resistance mechanisms in TB drugs. See the article titled “TB: Dangerous Comeback” (http://indiatogther.org/2012/feb/hlt-tb.htm) for more information about drug resistance in XDR-TB.
discovery of the antimicrobial drug. Antibiotic resistance was first observed in *Staphylococcus aureus* against penicillin in 1947, just four years after the mass production of the drug started. Since then, with the increasing use (and misuse) of a variety of antibiotics in both human and veterinary medicine, the process of development of antibiotic resistance has only accelerated. "More and more bacteria are becoming resistant to common antibiotics and to make matters worse, more and more are becoming resistant to all known antibiotics," laments the on-line journal *Science Daily*.

Now, what do antibiotics do? Antibiotics are designed to block some essential steps in the life cycle of the bacteria and prevent their growth and survival. These include synthesis of cell wall, folic acid, DNA, RNA and protein. Since many of these processes occur in the host cell (human and animal cells) also, targets chosen are specific to the bacteria, so that the drug may not harm the host cells. For example, unlike human and animal cells, bacterial cells have a thick cell wall. Antibiotics of the class beta-lactams (which includes penicillin) bind and inactivate an enzyme (called ‘penicillin binding protein’-PBP), which is essential for the synthesis of the cell wall. A bacterial cell without a robust cell wall cannot survive.

Folic acid biosynthesis is another example how these tiny bugs defy mighty human efforts. Folic acid is required by both bacteria and humans for the synthesis of nucleic acids and proteins. Unlike humans, bacteria cannot use pre-formed folic acid and synthesize their own folic acid. An important starting compound for the synthesis of folic acid is para-aminobenzoic acid (PABA). Sulfonamides and other sulfa drugs are analogous to PABA and bacteria cannot distinguish between the two. These compounds compete with PABA in biochemical reactions. When chosen, they block the synthesis of folic acid and thus the formation of nucleic acids and proteins, killing the bacteria.

An essential step in DNA replication prior to cell division is the unwinding of the double stranded DNA molecule. This is carried out by an enzyme called DNA gyrase. A class of antibiotics known as Fluoroquinolones bind to bacterial DNA gyrase and inhibit DNA replication, preventing bacterial growth. Rifamycins inhibit RNA synthesis in an analogous manner.

Ribosomes are structures on which protein synthesis takes place. Tetracyclines, Erythromycin and similar antibiotics bind to ribosomes to prevent protein synthesis.

Adapting by Mutating

Bacteria have great ability to adapt to hostile environments. They develop resistance to agents that threaten their survival. All these resistance mechanisms are generated through genetic modifications so that the progeny will also be resistant to the drug. These genetic modifications occur at two levels: mutations in the chromosomal genes and horizontal transfer of resistance genes from one bacterium to another.

There are many ways by which bacteria can defend themselves from hostile aspects of an environment. For example, bacteria can produce an enzyme that binds to the drug and makes it ineffective. Penicillin is deactivated through the production of beta-lactamase. Alternatively, the target itself may be altered so that the drug may no longer be able to bind to it. This can be seen in the other types of penicillin resistant bacteria where the structure of the binding site PBP is altered. If an essential metabolite is altered by the drug, the bacteria may stop needing that metabolite.

Resistance to sulfonamide arises when bacteria develop the ability to utilize the pre-existing folic acid rather than synthesizing it. And then some bacteria may reduce the permeability of the cell wall to the drug or increase the active efflux (pumping out) of the drug from across the cell wall so that it may not be available at a high enough concentration to be effective.

Antibiotics may also bind to DNA gyrase and cause DNA damage in the form of single and double-strand breaks. Bacteria also respond to this assault in an interesting manner. In a series of complex genetic maneuvers, the cell will be able to repair the damage and resume DNA replication. However, this process known as the “SOS repair” is error-prone; it can randomly substitute the wrong bases during DNA replication leading to gene mutations. Most of these random mutations are harmful to the bacteria. But some of them may be beneficial to the bacteria and provide an advantage to its survival by triggering a defense mechanism.
Adapting by Acquiring Genes

A process known as “horizontal gene transfer” can also confer drug resistance to bacteria. There are three mechanisms by which horizontal gene transfer can take place: conjugation, transformation and transduction.

Bacteria contain a DNA entity called the ‘plasmid’. Plasmids are circular DNA strands capable of replication, independent of the chromosomal DNA. A unique property of plasmids is that copies of replicated DNA can be transferred from one bacterium to another, sometimes even across the species. Known as conjugation, this process is akin to mating in higher organisms. When two bacteria come close to each other, a hollow bridge-like structure called the ‘pilus’ forms between them to facilitate a copy of the plasmid to move from one to another. Plasmids may contain genes that render the bacteria resistant to specific antibiotics. In such a case, the recipient bacteria also become resistant to that antibiotic.

Another means by which bacteria can acquire a ready-made resistance gene is a process called ‘transformation’.

Superstar Superbugs

Bacterial classes like staphylococci, enterococci, pneumococci all have the ability to become superbugs. There are strains of E. coli resistant to five variants of the drug fluoroquinolone!

The best-known superbug is the Methicillin-resistant Staphylococcus aureus (MRSA). Staphylococcus aureus first developed resistance to Methicillin – a penicillin-class antibiotic – in 1947. Later strains are reported to have developed resistance to a number of antibiotics like tetracycline, erythromycin, vancomycin, and linezolid. MRSA is commonly found on human skin and mucous membranes. It is easily contacted in places like gym, schools and hospitals. It is quite common in Europe, UK and USA. According to a report, in 2005 MRSA was responsible for nearly 95,000 cases of serious infection with almost 19,000 hospital-related deaths in United States. MRSA has also been successful in transmitting resistance genes to a completely different species of bacteria – Enterococcus faecalis – making it resistant to vancomycin.

Another bacterium – Streptococcus pneumoniae – has been a major cause of community-acquired infection such as upper respiratory infection, bronchitis, pneumonia, otitis media, pharyngitis and meningitis. Even though it was once almost eradicated by penicillin, it has now developed significant resistance to penicillin, trimethoprim sulfamethoxazole, macrolides, tetracyclines, and fluoroquinolones and thus has become a major problem.

Another well-known superbug that is bothering public health authorities in the developing world is Mycobacterium tuberculosis. It is reported that tuberculosis kills about 1.7 million people around the world, of which three to four lakh deaths occur in India due to the presence of resistant strains like Multi-Drug Resistant TB (MDR-TB) and Extremely Drug Resistant TB (XDR-TB). Now, Hinduja Hospital, Mumbai has reported the isolation of yet another resistant strain known as Totally Drug-Resistant TB (TDR-TB), which is found to be resistant to twelve drugs.

Recently, a new resistant strain of Klebsiella pneumoniae was detected in a Swedish patient of Indian origin. This produces an enzyme named New Delhi-metallo-beta-lactame-1 (NDM-1), which inactivates a broad range of beta-lactam antibiotics. Since this was first found in a patient who had undergone medical treatment in India, the researchers named it after New Delhi. The gene for NDM-1 can spread horizontally and at least twenty strains of bacteria, each resistant to one or many antibiotics, are now known.
**Feature Article**

**Antibiotic Resistance Mechanisms**

Folic acid is required by both bacteria and humans for the synthesis of nucleic acids and proteins. Unlike humans, bacteria cannot use pre-formed folic acid and synthesize their own folic acid.

**Fighting the Superbug**

Microbiologists have been working for decades on ways to combat antibiotic resistance. One of the fertile targets is the SOS repair pathway as preventing the induction of SOS repair reduces formation of drug resistance not only through chromosomal mutations, but also other mechanisms like horizontal gene transfer and homologous recombination.

However, some researchers feel that such attempts may only extend the time needed for resistance development and will not eliminate the problem completely. This is because antibiotic resistance is an unavoidable consequence of the indiscriminate use of antibiotics. According to the Food and Drug Administration of the USA, drug resistance “is an outcome of the Darwinian biological principle of ‘Survival of the fittest’”.

As long as the effectiveness of the drug is based on chemical processes, bugs can always develop resistance to the process. Hence, departing from the conventional approach, some researchers are trying to develop next generation antibiotics that may attack bacteria through physical or mechanical means.

Inspiration for these new approaches has come from the body’s own defense mechanism – the immune system. It is known that white blood cells engulf bacteria and rip them apart. An electrically charged protein known as “defensin” plays a major role in this process by binding to the bacterial cell wall and creating gaping holes through which the cellular contents flow, thus killing the bacteria. But there is a problem in harnessing them as antibiotic agents; laboratory-produced defensins are destroyed by the host’s immune system itself before they reach the infected area.

Scientists at the University of Pennsylvania are trying to overcome this problem by stripping down the molecule to its essential membrane-busting component so that they are undetected by the host’s immune system. Researchers believe that the complexity of the cell wall prevents the bacteria from developing resistance to defensins. Scientists at the IBM research center are developing organic nanoparticles that function the same way. These particles are so designed that they are physically attracted to the bacteria like a magnet, break through the cell wall and destroy them. Clinical trials are under way in China. Researchers hope that they can be put into soaps, deodorants, hand sanitizers and lotions.

Researchers at the Gamaleya Institute of Epidemiology and Microbiology, Moscow have some exotic technology in their kitty – cold plasma to kill the bacterial Plasma is an ionized gas that typically exists at very high temperatures. But the new tool produces plasma at 35 to 40 degree Celsius, easily tolerated by human skin, by exciting a gas like argon with RF radiation. They tried this torch against two bacteria that show up frequently in infected wounds – *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

In preliminary experiments on rats with infected wounds, exposure for ten minutes killed 90% of the bacteria. The plasma, interacting with the tissue releases reactive oxygen species that are lethal to the bacteria. The process also appeared to accelerate wound healing. The other encouraging factor is, these methods do not distinguish between resistant and non-resistant bacteria, but are equally lethal to both.

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Dr M.S.S. Murthy lives at B-104, Terrace Garden Apartments, 2nd Main Road, BSK IIIrd Stage, Bangalore-85