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|  | **THE SUPERBUG: A Warning Call** In 1995, the number of reported cases of food poisoning caused by Salmonella typhimurium had reached 3,500 - more than 23 times the number when it was first identified in 1984. In New York City, frustrated health professionals are battling several different strains of a disease long forgotten - a new form of tuberculosis that is resistant to all known drugs. In Japan, the Aichi Prefectural Institute of Public Health collected samples of the deadly Escherichia coli strains O57 and O26. Over a third of the samples were found to be completely resistant to antibiotics. Malaria is coming back strong in Africa to become one of the worst infectious disease episodes. In the next twenty years, hepatitis C is predicted to claim the lives of three times the current number of people infected with the disease. The list goes on...  It has only been seventy years since the door to the development of antibiotics was first opened by Sir Alexander Fleming with his amazing discovery of a penicillin-producing mold. Today, these antibiotics are all coming up short against mutating bacteria. Bacteria are generally classified into two groups: gram positive and gram negative. This type of classification originated from the Danish microbiologist Hans Christian Gram, who developed a stain to differentiate between the two groups. Gram positive bacteria are surrounded by a relatively thick layer of polysaccharide molecules and polypeptide chains. This layer of cell wall, usually 15 to 80 nanometers thick, enables the bacteria to better absorb the stain and will cause the bacteria to appear purple when viewed under the microscope. On the contrary, gram negative bacteria contain a much thinner layer of polysaccharide molecules and polypeptide chains. An outer membrane made up of lipopolysaccharides, polysaccharides chains with lipids attached, surrounds this layer. The double-layered cell wall prevents gram negative bacteria from picking up the stain; thus, such bacteria will lack the purple color of gram positive bacteria when viewed with the microscope. Due to the structural differences of the cell walls, the two types of bacteria are susceptible to different kinds of antibiotics. Many antibiotics work by inhibiting the protein synthesis of bacteria; since the cell wall of gram positive bacteria consists of polypeptide chains, it will be destroyed by the antibiotics, and the bacteria will burst. Protected by their outer membrane, gram negative bacteria would be unaffected by antibiotics functioning in this manner. Thus, gram negative bacteria will require other methods of treatment and are more difficult to control.  Though antibiotics worked efficiently in controlling most bacteria, the pathogens are rapidly developing means of defense to antibiotics, transforming into what is called the "superbugs". This resistance is then easily passed on to other bacteria through plasmids, circular, duplex molecules of DNA that are free to travel about. As their resistance to antibiotics grows, the drugs are found to be less and less effective. Professor of pathobiology at the University of Washington School of Public Health and Community Medicine, Dr. Marilyn Roberts states, "Until recently, if one drug became useless, there was always another [antibiotic] we could turn to. The problem is, the endless supply of new drugs has essentially stopped. There are very few new classes of antibiotics being developed. As a consequence, we have some diseases now for which no treatment is 100% effective" (www.hslib).  Defense mechanisms usually emerged as a result of the misuse of medication. Antibiotics have apparently been prescribed too frequently, and many widespread uses of them are questionable. In many Third World countries, there are often no need for prescriptions for antibiotics. Some patients do not finish taking their prescription, or would take medication prescibed for someone else. These factors all contribute to the problem of antibiotic resistance.  Superbugs can cause for much frustration and life-threatening situations. When a normal treatment of antibiotics for a certain bacterial infection no longer works, a new treatment must be created. Such a process may take up too much time and may not be developed in time to save the patient. The worst news is that there appears to be many strains of bacteria erupting at the same time that are resistant to antibiotics. This can eventually lead to a world-wide plague that may be uncontrollable. "I think we've gone about as far as we can go in terms of developing new antibiotics," said Herbert L. DuPont, M.D., director of the Center for Infectious Disease at The University of Texas-Houston Health Science Center, "In the future, we'll see more focus on the patient and ways to boost the patient's immune system and resistance to infection" (www.better). As a result, health professionals are frantically trying to come up with alternatives to antibiotics; and it is in this direction that our experiment is headed.  **GARLIC: An Ancient Remedy**  Throughout history, garlic has been a very important and highly treasured herb to mankind. Originated in western Asia, garlic was believed to be brought to Egypt by nomadic tribes, then to India through trade routes, and finally to Europe. It is greatly valued to every civilization ranging from the Ancient Greeks to the Chinese. The Babylonians reserved a special place for garlic at the table of their God-king, while the Vikings always brought the herb along with them on their voyages for food. In Ancient Egypt, fifteen pounds of garlic could be traded for a strong male slave. Besides being a source of food, garlic was used for magic and medicine.  The Ancient Greeks were known to worship Hecate, the under-world goddess of magic, charms, and enchantment, with garlic placed on piles of stones. In addition, a giant garlic sits near the Parthenon to protect the gates of Athens from the mythical nymphs. Hippocrates, known as the Father of Medicine in 460 B.C., used garlic to treat infections and epilepsy, as well as wounds, toothaches, chest pains, and intestinal disorders. Aristotle had said that "[Garlic] is a cure for hydrophobia and tonic, is hot, laxative, but bad for the eyes" (Harris, 50). However, the Greek aristocracy disliked the herb for its offensive smell.  In Ancient Rome, soldiers were constantly fed garlic for strength and courage on the battlefields. It was also believed to be a powerful aphrodisiac. A Roman naturalist, Pliny, was an active supporter of garlic's medical uses and the author of Natural History, which included sixty-one garlic remedies for respiratory ailments, blisters, ringworm, wounds, and other infections. Unfortunately, the foul-smelling herb was again rejected by the wealthy, and laws were passed by the Roman Senate to forbid those entering the Temple of Cybele, Mother of Zeus, from using garlic.  When Marco Polo traveled to China in the thirteenth century, he was perhaps surprised to observed that:  The poore sort go to the Shambles and take the raw liver as  soon as it is drawn from the beasts; then they chop it up small,  put it in garlic sauces and eat it there and then. And then they do  likewise with every other kind of flesh. The Gentry also eat their  meat raw. (Harris, 62)  Besides using garlic as a spice, the Chinese used it in preserving fresh meat and as "a masking agent for meat and fish past their prime" (Harris, 62). They also knew of its beneficial effects in treating the spleen, stomach, and kidneys. In fact, garlic had an important role in the ancient Chinese moxa treatment, or moxibustion. The treatment consists of applying burning moxa cones at various acupunctual points on the body, and garlic is often placed between the cones and the skin. Heat from the moxa cone travels through to the garlic and mixes with the chemicals of the herb. The heat then travels to the skin and is found to be a successful treatment for many respiratory ailments including asthma and tuberculosis.  The most significant evidence of the use of garlic by ancient civilizations is found in the Codex Ebers. Discovered by the German Egyptologist, George Ebers, the Codex Ebers is the world's oldest surviving medical text containing medical work dating back to 1550 B.C. The papyrus is a collection of over eight hundred remedies, of which twenty-two are garlic-based. Garlic's significance to the world before antibiotics is irrefutable. It had been used from the beginning of mankind in treating ailments ranging from the common cold to snakebites. Some interesting remedies and uses include:  \* Garlic diluted in water and applied as a tonic to the scalp is used for graying hair in India.  \* A clove of garlic is placed inside a woman's womb during the night until dawn in Egypt. If the smell of the garlic is in her mouth, then she will become pregnant.  \* For the Native Americans, garlic wrapped with salt and wool is put in the ear for earaches.  \* It is believed that a wild garlic remedy had saved Marquette's party from starvation during their journey to the Great Lakes. He then named his party's campsite, Cigaga-Wunj (place of the Wild Garlic), and it eventually became what is now Chicago.  \* During the Black Plague of the Middle Ages, some herbalists were known to have worn garlic around their necks and eaten large amounts of garlic. Surprisingly, they did not contract the contagious disease. To this day, no one can be sure whether garlic had certain chemical properties that helped protect against the plague or whether its offensive smell kept others away from them so that the infection was not spread.  \* Dogs were treated for rabies in the American Southwest by adding garlic into their food.  **GARLIC: From a Scientific View Point**  Perhaps as scientific look at garlic can explain mankind's fervor and beliefs with the "wonder bulb." The scientific name for garlic is Allium sativum; also included in the Allium genus are chives, onions, leeks, and shallots. The garlic plant consists of a bulb at the base and a flower stalk that rises directly from the bulb. At the very top is usually a globe-shaped white flower head. The bulb itself is surrounded by many layers of white skin and is composed of individual sections, or cloves.  Garlic is a mineral-rich perennial plant, and the following analysis from a Japanese Standard Food Component Analysis (Harris, 133) will give a more detailed look at the contents of the bulb:  MINERAL MATTER / VITAMIN CONTENT   |  |  |  |  |  | | --- | --- | --- | --- | --- | | Calcium | 18 mg |  | Vitamin C | 20 mg | | Vitamin A | 16 I.U. |  | Iron | 1.7 mg | | B2 | 0.08 mg |  | B1 | 0.22 mg | | Phosphorous | 67 mg |  | Nicotine acid | 0.4 mg | | Carotin | 50 I.U. |  |  |  |   \* Based on 100 grams of garlic  In addition to having vitamins and minerals, garlic has a high sulfur content. In the late 19th century, German scientists extracted allyl compounds (diallyl disulfide) from garlic, and in the 1940's, Dr. Arthur Stoll discovered the presence of alliin, which was later identified as S-allyl-L-cysteine sulfoxide. When a clove of garlic is cut or crushed, the enzyme alliinase is released and alliin is converted into allicin. It is this yellow, oily liquid that is responsible for garlic's strong, offensive smell. Allicin, with a chemical formula C6H10OS4, has the unique ability to block two groups of enzymes, cysteine proteinase and alcohol dehydrogenase, that are essential to dysentery-causing amoebas.Cysteine proteinase is the main cause of infections, providing bacteria the means to invade and damage tissues; alcohol dehydrogenase is important to the metabolism and survival of infectious organisms. Researchers are, thus, slowly drawing the conclusion that allicin is responsible for the antibacterial action of garlic. To block the enzymes, allicin reacts with the sulfhydryl groups of the enzymes. Since sulfhydryl groups are essential to some enzymes involved in the synthesis of cholestorol, allicin may also be able to lower the levels of LHL, the primary substance leading to damages of the arterial walls. In addition, researchers are suggesting that allicin acts as an antioxidant, which destroys the agents believed to cause tumor growths. Though the chemical can be destroyed by heat and may dissipate within hours after a clove is crushed, allicin was found to be more effective than penicillin in treating typhus disease, strep, staph bacteria, and organisms that cause cholera, dysentery, and enteritis. In 1944, garlic was found by Calvalitto et al. to be effective against both gram positive and gram negative bacteria. In 1954, a Russian researcher, T.D. Yanovich, performed an experiment by placing garlic juice directly into bacterial colonies. He found immobile bacteria present within two minutes, and activity of the bacteria ceased within ten minutes. All research seems to suggest that garlic may be an alternative to antibiotics.  **OUR TEST SUBJECT: Bacillus cereus**  Researchers had discovered that the Bacillus anthracis is the most sensitive bacterium to garlic. Because the Bacillus anthracis produces the poison anthrax and may be deadly, we turned to a less harmful relative of the B. anthracis, the Bacillus cereus to serve as the test subject of our experiment.  Bacillus cereus is a species of spore forming gram positive motile rod bacteria that can cause many clinical syndromes. The emetic syndromes, which result after only a short incubation period (1-6 hours), include nausea, vomiting, and abdominal cramps. After a longer incubation period (6-24 hours), diarrheal syndromes will begin to be present. The bacterium can also cause local skin, wound, and ocular infections. Invasive diseases including bacteremia, endocarditis, osteomyelitis, pneunomia, and meningitis can be results from an infection of the Bacillus cereus. Many of these syndromes are caused by a heat stable toxin produced by the bacterium. The diarrhea syndromes, however, are caused by labile enterotoxin, which is a toxin produced by the bacterium that kills intestinal cells and cause the vomiting and diarrhea associated with food poisoning. The ideal temperature for the bacterium to grow and produce its toxins range from 25(C (77(F) to 42(C (107.6(F). Normally, small numbers of the bacteria are present in raw dried and processed foods; its diseases are acquired by eating foods with its toxin or spores. As a result, a Bacillus cereus infection is generally classified as food poisoning. The spores of Bacillus cereus are heat resistant and can survive brief cooking or boiling. A Bacillus cereus infection is not transmissible from person to person. When a person is diagnosed with having a Bacillus cereus infection, oral rehydration (drinking a lot of water) is normally suggested. If the victim has a severe case of the infection, then antibiotics are prescribed. Aminoglycosides, chloramphenical, elindamycin, ciprofloxacin, erythromycin, imipenem, and vancomying are all antibiotics that Bacillus cereus is susceptible to. To kill the bacteria, these antibiotics either inhibit its protein synthesis or break down the bacteria's thick cell wall.  **Antimicrobial Susceptibility Test**  In order to test the effects of garlic on bacteria, we must perform the antimicrobial susceptibility test. This type of testing is used to predict the success or failure of certain antibiotic treatments and is usually performed in vitro, or in a laboratory setting.  One of the more commonly used methods of antimicrobial susceptibility testing is the Bauer-Kirby Disk Diffusion. Small disks of an absorbent material, such as filter paper or chromatography paper, are soaked in antibiotics and placed directly onto a bacteria-rich agar plate. Zones of inhibition around each disk should be produced after the plate had been incubated overnight. These zones of inhibition provide the means of measuring susceptibility, dividing the organism into one of three categories: susceptible, intermediate, or resistant. Because each strain of bacteria is different, there are different ranges of values for the susceptibility of each strain to each antibiotic. It should also be noted that the sizes of the zones of inhibition may vary depending on factors such as: medium base, humidity, and the age of the medium. However, it can generally be stated that the larger the zone of inhibition, the more susceptible the organism is to the antibiotic.  Our experiment has been designed to test for the susceptibility of the Bacillus cereus to garlic. This information will then be compared to the susceptibility of the Bacillus cereus to an antibiotic. If the levels of susceptibility are similar, then we may be able to conclude that garlic can be an alternative to the antibiotic in treating the Bacillus cereus. |

*This Web Site is Best viewed with 256 or more colors.*

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