



## CRITICAL FOCUS

Brian J. Ford

### Crisis Point: The Rise and Fall of Penicillin

Our lives are at risk. The World Health Organization (WHO) says there is a global security threat that requires action across government sectors and society as a whole. In Atlanta, the Centers for Disease Control and Prevention (CDC) are similarly blunt, describing the situation as a nightmare. In London, Chief Medical Officer Sally Davies, says we now face a catastrophic threat. What is it? International terrorism? No, says Davies, the danger is greater than terrorism.

The CDC adds that it is a “critical health issue.” So is it Ebola? Bird flu? SARS? No. Their cause for concern is something far more insidious: bacterial resistance to antibiotics. Everywhere in the world, bacteria are resisting our treatment and the problem is spreading. Doctors in the U.S. tell of patients — several each year — whom they diagnose with a simple infection but who cannot be treated by any of today’s drugs. Just five years ago, such patients were so rare they were an academic curiosity yet now they are appearing in doctors’ consulting-rooms. After decades of improvement, medicine is losing its grip.

Some staphylococci are now unaffected by drugs that once eliminated them, and now among the recent resistant bacteria to emerge we have *Escherichia coli* and *Klebsiella pneumoniae* which we have long regarded as minor irritants but which are suddenly killing patients

---

*Everywhere around the world, bacteria are developing a resistance to antibiotics. Patients with a simple infection cannot be treated by any of today’s drugs.*

---

as never before. Standing by as a patient succumbs to what was once a trivial infection is a devastating experience for any physician; and this is becoming more common as the weeks go by. In the 1940s,

the world of medicine was deliriously happy as penicillin was introduced, and once-fatal diseases were quickly cured with a course of capsules. Now, the picture is very different, and methicillin-resistant *Staphylococcus aureus* (MRSA) is now widespread.

Bacteria have long been familiar objects of study. Microscopists understand what goes on beyond our normal sight. This explains why you can always identify the microscopists at a conference — we always wash our hands *before* we take a wee, not afterwards. Your private parts have been tucked in your underpants out of harm’s way, but heaven alone knows what might have been picked up on our fingers. We microscopists are instinctively aware of the microscopic realm everywhere we go, which is why we live longer than most people.

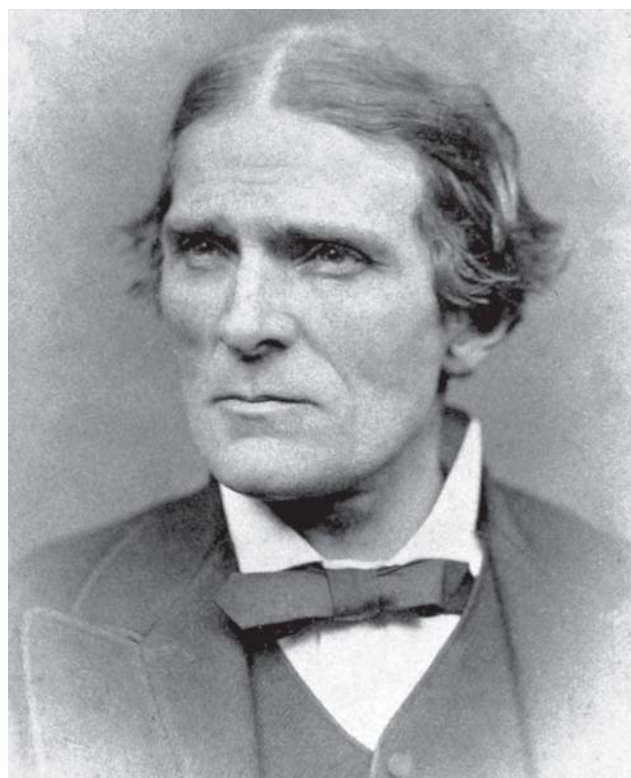
The first person to observe the effect of penicillin was not Alexander Fleming — it dates back much further in history. I have often wondered whether the bread poultice traditionally applied to surface lesions may have become popular because of antibiotics produced by mildew growing in the stale bread that was used. The first formal recognition of the role of

*Penicillium* dates back to London in 1871, when Sir John Burdon-Sanderson noted that thick bacterial growths quickly formed if broth were exposed to air, but if a mold like *Penicillium* grew on the surface of the broth then the bacteria died down. That same year, the pioneering English surgeon Joseph Lister recorded that samples of urine contaminated with the mold would not support the growth of bacteria. Lister went on to introduce antiseptics like carbolic acid into routine medicine.

Sir William Roberts published *A Practical Treatise upon Urinary and Renal Diseases* in 1872, and two years later he noted that broth cultures of *Penicillium* remained free from bacterial contamination. Two years after that, the same effect was observed by John Tyndall. Then in 1877, Louis Pasteur and Jules Francois Joubert in France observed the inhibition of growth in a culture of anthrax bacteria when contaminated by *Penicillium*, a phenomenon that Pasteur and Robert Koch named “antibiosis.” A further step along the road came in 1897, when a French medical student named Ernest Duchesne published an account of the inhibition of bacteria by *Penicillium* fungi. He concentrated the antibiotic in broth and used it to show that it could be used to treat animals infected with bacteria — but he died in 1912 at the age of 37 and nobody took his findings any further. In Paris in 1923, a bacteriologist from Costa Rica, Clodomiro Picado Twilight, recorded the “inhibitory action of *Penicillium* sp.” on staphylococci. He was working at the Institut Pasteur but could interest nobody else on his discoveries. This is a remarkable revelation — I have found at least 10 investigators who had noticed the effects of penicillin before Fleming.

## EARLY “ANTIBIOTICS”

The term “antibiotic” was already in the literature by this time, though in a very different context. A U.S. meteorologist who had served in the navy, Matthew F. Maury, coined the term in 1855 in his book entitled *Physical Geography of the Sea and its Meteorology* but at the time this term had nothing to do with medicine. Maury was an enthusiastic observer of the heavens, and wrote about the possibility of there being life in outer space. He was a sceptic and expressed his view by concluding that “I incline to the antibiotic hypothesis” — there was no alien life out there in space. A French microbiologist first used the term in its modern sense. In 1890 Pierre Vuillemin defined an “antibiotic” as any substance that was “injurious to or destructive of living matter, especially microorganisms.” So the history of the antibiotic era is already proving



The action of penicillin was not first discovered by Alexander Fleming but by John Burdon Sanderson (pictured), a British physiologist from Newcastle-upon-Tyne in the north of England. His crucial observations in 1871 were confirmed by Joseph Lister, who went on to introduce carbolic acid as an antiseptic for use in operating rooms.

to be very different from what we tend to imagine: A number of scientists had observed the effects of antibiotics in the 19th century, dating back before Fleming was even born.

Prior to the era of antibiotics we had sulfonamides that were developed in Nazi Germany. Unlike antibiotics (which are produced by microbes) the so-called sulfa drugs were a byproduct of the coal tar industry and were first researched by the Bayer Company. The first successful antimicrobial drug was Prontosil, a red dye discovered by Dr. Gerhard Domagk at the IG Farben works. These sulfa drugs could kill streptococci, and Domagk tried the new drug out on his daughter who had a severe infection and was due to have her limb amputated. The sulfa saved her. Domagk's discovery was patented in 1933, just as the Nazis came to power, but nothing was published about it for years, so great was the sense of secrecy. A range of similar drugs was soon available.

BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY, VOL. X, NO. 3.

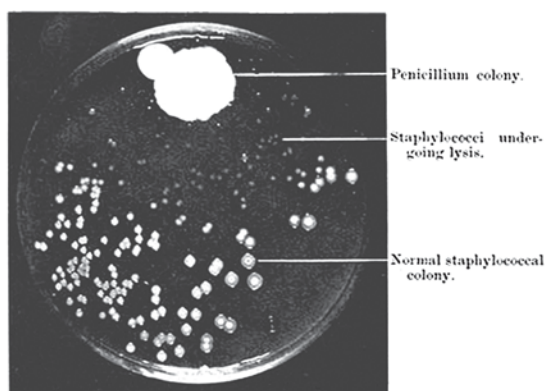
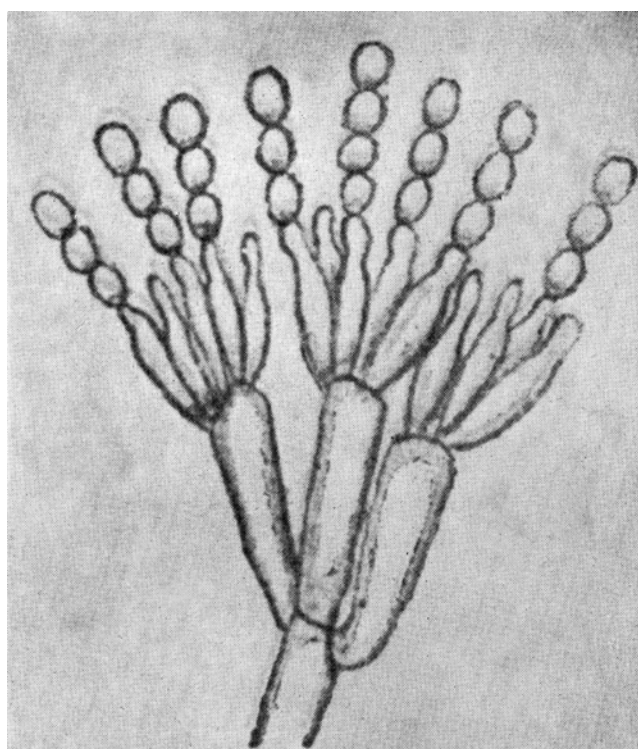


FIG. 1.—Photograph of a culture-plate showing the dissolution of staphylococcal colonies in the neighbourhood of a penicillium colony.

More than 50 years after Sanderson's observations, the action of *Penicillium* on bacterial cultures was photographed by Fleming in 1928. This is his original image with the captions he produced for publication. Fleming continued to maintain the fungus in culture and was able to offer samples to other investigators.



The sporing bodies of *Penicillium* have branching hyphae that spread like bristles in a brush (the Latin for paintbrush is "penicillum"). This phase contrast micrograph shows the branching of the hyphae as sporangia form. *Penicillium* is a saprophytic ascomycete, which can itself cause opportunistic infections in people who are immunologically compromised.



When Fleming first worked on his mold he incorrectly identified it as *Penicillium rubrum* and only later did he recognize it as *P. notatum*. This species had been discovered in 1911 on a pile of decaying hyssop (a fragrant herb) by Swedish mycologist R.P. Westling in Scandinavia. Westling made this first pencil drawing of the fungus.

These sulfa drugs were the first major development in antibacterial therapy and deserve their place in the history of medicine. Only arsphenamine, an early antimicrobial developed by Paul Ehrlich in 1909 and used to treat syphilis, had gone before. Arsphenamine (marketed as Salvarsan) was an effective anti-syphilitic, but it also caused liver damage and other severe side-effects. Although the sulfa drugs were important, they were limited in scope, and it was penicillin that would provide the first safe and reliable treatment for a range of common bacterial infections. Today it remains one of the most widely-used antibiotics. That, however, is about to change.

### FLEMING'S PENICILLIN

The saga of penicillin is an episode of scientific history that has yet fully to be told. It began on the morning of Sept. 28, 1928, when the recently appointed professor of bacteriology, Alexander Fleming, broke his vacation and came back to London unexpectedly to help a colleague. He used some spare time in his laboratory sorting out petri dishes bearing cultures of *Staphylococcus* bacteria. Professor Merlin Pryce called in to speak to Fleming, and one of the dishes caught their attention, for a large colony of greenish mold had taken up residence and around it no bacteria would grow. Fleming wrote that the bacterial colonies around the mold colony had "dissolved away." The fungus turned





Fleming provided cultures of his mold for microbiologists who requested them, and in 1935 he presented one to Douglas Macleod, who had recently joined the staff of St. Mary's Hospital in London. Macleod had it mounted in this splendid wooden and brass case, and in June 2014, it was sold at auction in London for more than \$25,000.

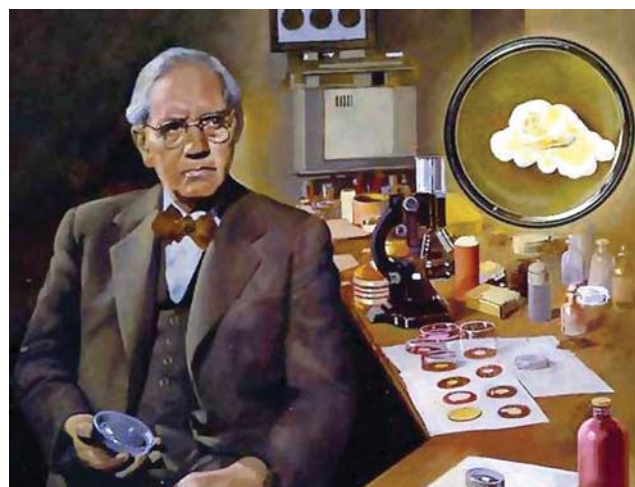
out to be *Penicillium notatum*.

Fleming had worked for years on bacterial infections. In 1921, he observed that nasal mucus and tears could prevent the growth of bacteria and he discovered they contained an active agent he named lysozyme (1,4- $\beta$ -N-acetyl-muramidase). Lysozyme is an enzyme that dissolves bacterial colonies, which explains why Fleming thought that had happened to the staphylococcal colonies on his petri dish. Later, he worked with a junior colleague, V. D. Allison, and they detected lysozyme in serum, saliva, even milk and other natural liquids. Additional tests showed that, although lysozyme dissolved many harmless types of bacteria, it was less effective against disease-causing species. We now know that penicillin acts differently to lysozyme, however; it inhibits growth, rather than dissolving existing bacterial colonies.

Where had the *Penicillium* colony come from? Accounts everywhere say that the mold spores that contaminated Fleming's agar plate must have wafted in through the open window, but Fleming kept the windows closed (they looked out over a busy road, Praed Street in Paddington), and the mold probably came from the mycology laboratory below Fleming's room, where these fungi were being cultured. When Fleming tested all their cultures for antibiotic-producing

THE ROYAL INFIRMARY, SHEFFIELD. F100			
No. <u>105</u>	Age <u>32</u>	Date <u>28.8.30</u>	
Name <u>Boles</u>	Occupation <u></u>		
Address <u></u>			
Disease <u>R. V. L.</u>	Abilities <u>hematoma</u>		
Pupils <u>R</u>	Tension <u>L</u>	Vision <u>L</u>	
Out-Patient Number		TREATMENT.	
Past History			
<p><i>Purulent Discharge + + +</i>  <i>some time after birth</i>  <i>Treatment previously nothing.</i></p>			
On Admission			
<p><i>Some mucus &amp; irritation + + + R.</i>  <i>Como Chaggy R + L.</i>  <i>Culture Gonococci +</i></p>			
<p><i>Just Aug</i>  <i>not H. B.</i>  <i>Just black</i> } 2 hourly.</p>			
<p><i>25. 30. 30. 30. 30. 30. 30. 30. 30. 30.</i>  <i>2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.</i></p>			

Cecil Paine was the first to treat patients with penicillin. These unpublished records from 1930 were retrieved by Milton Wainwright and Harold Swan. Although appointed a lecturer in bacteriology at Sheffield University, Paine is not mentioned anywhere in the official history of the school.



Alexander Fleming was often portrayed in an idealized and heroic stance, and this Spanish painting by Biografias y Vidas is typical of the genre. After publishing his results, Fleming carried out no additional research into antibiotics. Once Howard Florey and Ernst Chain of Oxford University had announced their penicillin research, Fleming made himself readily available to the media.

strains, he found only one — and it was identical to the fungus that had already contaminated his petri dish. At that time Fleming had two assistants, the newly qualified Dr. Stuart Craddock and Frederick Ridley, so he asked them to try to isolate pure penicillin from the mold. It proved to be an unstable compound, and when Harold Raistrick, professor of biochemistry at the London School of Hygiene and Tropical Medicine, tried to purify the penicillin, he did not succeed.

Yet penicillin was used to treat patients many years before it existed in purified form. In 1930, crude penicillin was tested at Sheffield Royal Infirmary in England by a young doctor named Cecil Paine. He had studied under Fleming in London, and wrote to him to obtain a culture of the *Penicillium* fungus. Paine was concerned about *syphilis barbae*, a chronic infection of the beard follicles, but was unsuccessful because the solution could not penetrate the skin. He then tried to treat *ophthalmia neonatorum*, an infection of the eyes of newborn children caused by gonococci, and successfully cured his first case on Nov. 25, 1930. He subsequently treated four other patients with eye infections.

It has been rumored that one of Fleming's friends, a member of the St. Mary's Hospital marksmanship club, developed pneumococcal conjunctivitis about this time, and Fleming administered some of the partly purified mold extract to cure him. In another case, Fleming once said he used the penicillin-containing broth to cure indolent ulcers of a woman patient. Stuart Craddock, meanwhile, tried to treat his own chronic sinusitis, without effect. And after that, the research came to a halt.

There have since arisen many legends about Fleming's early days. One tells how the young Alec Fleming, at home on the farm in the Scottish lowlands, saved the life of Winston Churchill as a boy after he nearly drowned in a lake; another version has the Churchills marooned in mud in their horse-drawn carriage only to be rescued by the young Fleming. Lord Randolph Churchill, Winston's father, was said to be overcome with gratitude and paid for Fleming's medical education in London. A report from 1943 has Winston suffering from pneumonia, and being treated with the new wonder drug by Fleming who flew into North Africa on a life-saving mission. So it is said that on two separate occasions, Alexander Fleming rescued Winston Churchill.

Neither account is true. Turn to an article by Arthur Gladstone Keeney in *Coronet* magazine. In the December 1944 issue on pages 17–18 he published a fictitious tale titled, "Dr. Lifesaver." The "drowning" story was Keeney's invention and, although Churchill

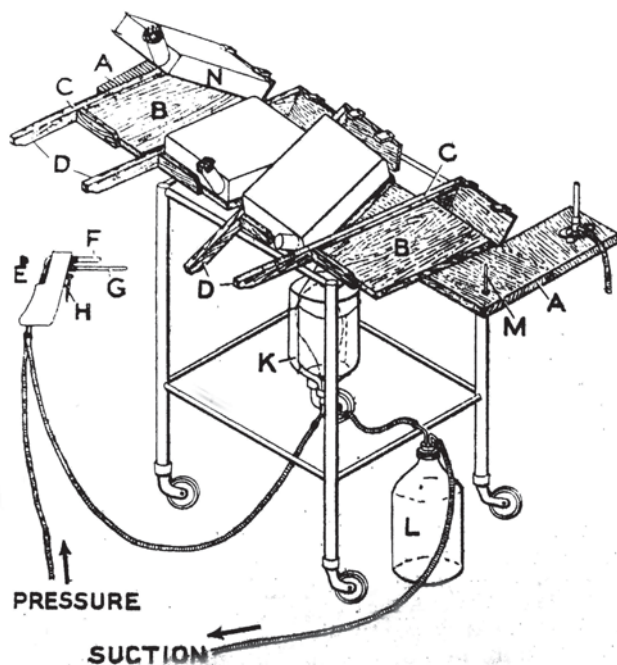
had suffered from a chest infection during the North Africa campaign, it was never treated with penicillin. The drug used was sulfonamide produced by the British company May & Baker. Known simply as M&B, this British version of the German discovery proved to be important in the management of acute infections during the war, and Churchill's experience served further to boost the drug's popularity. Keeney's fictions, meanwhile, entered the standard literature and they became widely repeated.

Fleming often wrote about the value of penicillin as a means of killing off competing bacteria in mixed cultures grown in petri dishes but, curiously, he hardly touched upon its use as a drug for curing infections. His first paper appeared in 1929 in the *British Journal of Experimental Pathology*. Its title, "On the antibacterial action of cultures of a penicillium," sounds prescient, but then it continues "with special reference to their use in the isolation of *B. influenzae*." That was how Fleming saw his discovery — as a means of damping down the opposition so that microbiologists could study their chosen species of bacteria.

## GLOBAL EFFORTS

The first person to see the true potential of penicillin in medical practice was Harold Raistrick, who had tried to purify the antibiotic. He was further frustrated by asking medical colleagues to assess the uses of penicillin as a drug; Raistrick failed to persuade them to look further into it. Little more was done until the pressures of war focused attention on the need to find a way of curing soldiers of their battlefield infections. Far more soldiers were wounded than killed, and they took many months to recover from infections resulting from war wounds. If a new drug could be found, it would give any nation an immediate advantage over Germany in the conduct of war.

American research in this field has been overlooked, but microbiologist Dr. René Dubos had isolated tyrothricin in 1939. He was raised in Hénonville, a small farming village north of Paris, and in 1927 he had joined Professor Oswald Avery's laboratory at The Rockefeller Institute for Medical Research in New York. Avery was searching for an antimicrobial enzyme in the war against pneumonia, and Dubos cracked the problem by identifying antibiotic activity in extracts of the soil bacterium *Bacillus brevis*. His analysis proved that tyrothricin was composed of two different antibiotics, 80% tyrocidine and 20% gramicidin. These were the first antibiotics to go into commercial production. Dubos published a little-known review titled



Trying to grow the *Penicillium* fungus in bedpans had not been successful, so N.G. Heatley devised a stackable ceramic culture vessel from which penicillin could be routinely harvested. Florey and Chain at Oxford created this system for mass-producing cultures: Heatley's flasks are labeled N, and the supernatant was collected in the flask labeled L.

"Microbiology" in the *Annual Review of Biochemistry* (Vol. 11, pp 659–678, July 1942). Even at that early date, he was writing: "During the past decade there have been studied a number of entirely new types of antibacterial substances ... prepared from microbial cells."

The Soviets were also investigating antibiotics. Dr. Zinaida Ermol'eva began working on penicillin at the Rostov Institute of Bacteriology in 1942, the same year in which husband-and-wife team Dr. Georgii Frantsevich Gause and Dr. Maria Brazhnikova discovered gramicidin. The first clinical use of this antibiotic in Soviet hospitals dates from 1943, and by the end of the war it was being used in front-line treatment of the wounded. Gause was presented with the Stalin Prize for Medicine in 1946.

The date when Tyrothrycin was discovered, 1939, was the same year in which an Australian scientist Dr. Howard Florey and Dr. Ernst Chain, a German-Jew who had escaped Nazism, began their studies of penicillin. Both were based at the Sir William Dunn School of Pathology at the University of Oxford. They were searching for mechanisms of antibacterial action, and

penicillin was attractive to these men for three reasons: Chain, as a biochemist, was fascinated by the behavior of this novel molecule; Florey was intrigued by its proven action against staphylococci, and both were diverted by the fact that Fleming still had cultures of the original fungus in culture at his laboratory in London. Chain set to work to extract crude penicillin from the broth in which the mold could be grown, and he diligently demonstrated that it was non-toxic to mice. Chain was not licensed for animal experimentation, so these toxicity experiments were carried out by Professor J.M. Barnes, who was later appointed as the first director of the toxicology unit at the Medical Research Council (MRC). Barnes confirmed what Fleming believed, namely that the supernatant was harmless to rabbits and did not damage leukocytes.

Fleming regarded penicillin as an aid to bacteriologists trying to prevent their cultures from being overgrown, but Chain clearly had in his sights a different idea: a super-drug. Florey realized that they would need specialist collaborators to carry the project forward and enlisted the cooperation of Dr. N.G. Heatley, who was in the same department. Heatley devised a technique in which small cylinders containing antibiotic solutions, each about the size of a cigarette butt, were stood upright on an agar plate. As the antibacterial agent diffused out of the cylinder, bacteria growing nearby were killed. This became the standard technique for assaying new antibiotics.

The team was then joined by another member of the staff, Dr. A.G. Sanders, who further developed this technique while Heatley worked on designing a culture dish for producing more of the mold. The first attempts to produce *Penicillium* in bulk were hampered by a lack of a suitable container. Petri dishes are not made for the mass-production of microbes so the team grew their first bulk samples of the mold — in bedpans. However, those were in short supply so Heatley designed a new form of flat-bottomed stackable china dish, which was used to produce penicillin-containing broth in quantity for the first time, using solvents to extract the antibiotic in a purified form.

At last they could carry out experiments to confirm that penicillin was truly capable of curing disease. Heatley dosed two groups of mice with streptococci and injected half of them with the first supplies of purified penicillin, leaving the others as controls. All the untreated mice died, but most of the treated mice survived. Florey was now eager to find out more of the pharmacology of penicillin extracts, while Dr. M.A. Jennings carried out tests that confirmed the penicillin solutions seemed to be harmless to human leu-



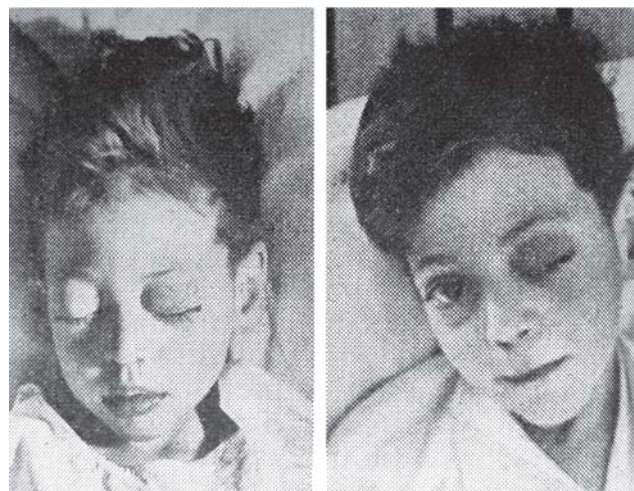
kocytes. The penicillin in the extracts they used must have been extremely dilute, so it is fortunate that there were no toxic components in the brew or penicillin might never have been developed.

## HUMAN TRIALS

Over the next few weeks, sufficient concentrated penicillin was collected for a trial in a human volunteer. The drug had proved to be harmless to animals, and also to human blood cells, but what would be the effect of treating a patient? A part-time police officer, Reserve Constable Albert Alexander, was close to death from an overwhelming staphylococcal infection in the nearby Radcliffe Infirmary. Dr. Charles Fletcher, who later became a popular BBC television presenter on medical matters, agreed to try and treat Alexander with the first batch of the purified penicillin. On Feb. 12, 1941, they administered 160 mg of the antibiotic. Within 24 hours, the patient's temperature was near normal and his appetite had returned. The treatment continued until they ran out of penicillin — their entire supply had been exhausted. Florey and his team managed to extract penicillin excreted in Alexander's urine, but as their supplies dwindled his infection returned and he slowly lost his fight for life. He died on the morning of March 15, 1941. The team Florey had set up thought it best to concentrate on treating children, because they would not need such large amounts of the drug. The first were carried out in the Radcliffe Infirmary by Dr. Fletcher working under Florey's supervision — and the results were spectacular.

Two central themes presented themselves; extracting pure penicillin and eliciting its chemical structure. At the Dyson Perrins Laboratory in Oxford, Dr. E.P. Abraham had just completed his Ph.D. in organic chemistry, and he resolved to tackle these problems. He was the first to identify the chemical structure of penicillin. It proved to be a  $\beta$ -lactam structure, though Abraham could not convince his former department head Dr. Robert Robinson, who believed it was a thiazolidine-oxazolone. The controversy was finally resolved by Dr. Dorothy Crowfoot (later Hodgkin) who used X-ray crystallography to confirm the existence of the lactam molecular structure. Among Hodgkin's later students was the young Margaret Roberts, who worked on the unraveling of the molecular structure of the Russian antibiotic gramicidin. This brilliant young student went on to leave science for a career in politics and, as Margaret Thatcher, became the prime minister of Britain.

At the Dyson Perrins Laboratory in 1941, Robinson



Florey and Chain published a pioneering paper on penicillin in *The Lancet* on Aug. 16, 1941. The article tells of this 4-year-old boy, who was severely ill on May 13, 1941; after receiving penicillin he seemed well by May 22, when treatment stopped. He later became ill and died on May 31. The cause of death was an aneurysm, unrelated to the infection.

had convinced himself that the best way ahead would be to chemically synthesize the antibiotic on an industrial scale, whereas Florey was certain that biological production from mold cultures would be the most propitious principle of production. Florey was right, and this remains the basis of antibiotic production to this day. I discussed this with Florey when we met at Oxford during a break from the Royal Microscopical Society's conference at the university in 1963. Florey had found that Heatley's culture dishes were never going to allow for the large-scale production of the new wonder drug, and soon the team were culturing *Penicillium* in glass milk bottles, which were readily available in large numbers. It was an amateurish approach.

Florey and Heatley realized that they should bring in the expertise of American scientists and they took their cultures to the U.S., where they discussed their discoveries with scientists at the Northern Regional Research Laboratory in Peoria, IL. Discussions centered on the nature of the mold: If *P. notatum* could produce penicillin, might there be other species that could produce more? One warm day, a laboratory assistant, Mary Hunt, arrived with a cantaloupe melon that she had purchased in the market that showed what she described as a "pretty, golden mold." The team identified it as *Penicillium chrysogenum*, and it produced 200 times as much penicillin as Fleming's original culture. Eventually they created mutations with X-rays and found



Margaret Hutchinson Rousseau, a leading American chemical engineer, designed the equipment used to mass produce penicillin. Dr. Rousseau graduated from M.I.T. and became the first woman admitted to the American Institute of Chemical Engineers.

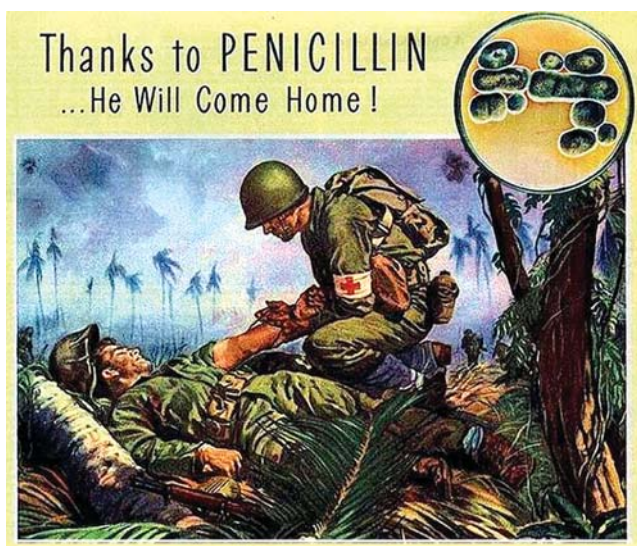
a variety of the fungus that could produce 1,000 times as much penicillin as the original culture of *Penicillium notatum*.

## PENICILLIN DEBUTS

In the U.S., penicillin was first released by Merck & Co. in 1942, and the first patient to be treated in America was Anne Miller, who was near death in New Haven Hospital in Connecticut after suffering a miscarriage and developing septicemia. Treating Miller consumed half the total supply of penicillin in America at the time. Within a year, there was enough of the antibiotic to treat 10 patients, and in July 1943 the War Production Board announced that manufacture would move ahead at maximum speed. By chance, an industrial byproduct proved to boost production. This was corn steep liquor, resulting from the milling of maize, and *P. chrysogenum* grown in this medium could be cultured in large amounts in steel fermentation vats much like beer in a brewery. The technique was the brainchild of Dr. Margaret Hutchinson Rousseau, a distinguished chemical engineer who was the first woman to become a Member of the American Institute of Chemical Engineers. This all happened in the nick of time, for it meant that there were 2.3 million doses in time for the invasion of Normandy on June 6, 1944. Within a year, more than 650 billion units of penicillin were being produced in the U.S. each month. It was a triumph of American enterprise.

Fleming's main contribution was the fact that he maintained his culture of *P. notatum*; otherwise he had nothing to do with this research. The development work on penicillin was spearheaded by Florey and Chain, and by the early 1940s Fleming had been all but forgotten. He has been written about as a "quiet and unassuming" man, though not everyone remembers him so. I discussed this 30 years ago with Monica Dobell, whose husband Clifford wrote the biography *Antony van Leeuwenhoek and his Little Animals* and whose father, W.S. Bulloch, published his momentous *History of Bacteriology* in 1938. Monica was lively and dynamic and spoke of her father as "Bully." Monica remembered Fleming well. She thought he was a jerk. "Horrid little man," she said. "Thought he owned the world with his penicillin, but Bully told me that he didn't do anything with it after his research around 1930." She didn't like Fleming? "He would sit in the room as if holding court," she reminisced. "Fleming was an unconscionable little oik."

He has been deified since. His laboratory at St. Mary's hospital, Paddington has been opened as a mu-



Schenley Laboratories promoted their penicillin with this dramatic advertisement, featuring an unrecognizable drawing of a microbe (upper right). Eli Lilly & Co. had already registered U.S. Patent No. 2,515,898 for penicillin production, and in 1953 the pharmaceutical company successfully sued Schenley for infringement.



seum of discovery with everything set out as it was in his day — with one exception. When I published a review of the exhibit in the *British Medical Journal* on Oct. 2, 1993, I mentioned an important omission: ashtrays. Fleming smoked cigarettes incessantly, and his laboratory bench always had ashtrays overflowing with spent cigarettes. The restored laboratory left them out of the display, claiming it might encourage the young to pick up the habit. It's an interesting dilemma: Should we falsify history to pacify political correctness?

Although Fleming had been left on the sidelines throughout the development of penicillin, when it became clear that a wonder drug was in the offing he began to speak to the press about his discoveries and liked to paint himself as the man who gave penicillin to the world. There was never any mention of his predecessors, and little said about the Oxford team who developed the drug. As the war ended, Fleming published a book titled *Penicillin* with some 30 contributions — yet not one of the Oxford team was among them.

Florey and Chain always emphasized that they were investigating antibiosis as a phenomenon and liked to say they were not searching for a super drug. They made the subject seem academic and detached. When I wrote about penicillin research in *Nature*, Chain was quick to respond and emphasized that they were not searching for a medicine. In the issue of *Nature* published on July 12, 1974 (p 98), I took him politely to task. All Chain's early joint papers hinted at therapy. The Oxford group may have wished to present themselves as detached and academic, but in my view there was no mistaking the subtext — they were on the trail of a super-drug and wanted the world to know.

## A CURE FOR TB

When the 1945 Nobel Prize in Physiology or Medicine was awarded, the rivals were finally brought together. The award went jointly to Sir Alexander Fleming, Ernst Boris Chain and Sir Howard Walter Florey “for the discovery of penicillin and its curative effect in various infectious diseases.” The world of science could sense that there were big bucks to be made from antibiotics and even greater reputations, so there was an immediate explosion of interest in research into antibacterial agents produced by microbes. Streptomycin was next to be discovered, and resulted from studies of *Streptomyces griseus* by an expatriate Ukrainian microbiologist at Rutgers University, Selman Abraham Waksman. Streptomycin was first isolated on Oct. 19 1943, by his graduate student

Albert Schatz, and its importance lay in the fact that it cured tuberculosis (an infection on which penicillin had no effect).

In 1946, the first patients were treated with streptomycin at the U.S. Army hospital in Battle Creek, MI. Their first patient died, and although the second was cured, he was left blind from a side-effect of the drug. Their third patient made a complete recovery in March 1946. He was Robert J. Dole, who was a presidential nominee and later became majority leader of the U.S. Senate. Meanwhile, some crucial randomized trials of streptomycin in treating tuberculosis were held in 1946–1947 in London by the MRC. These were the first ever double-blind trials in medical history and were so successful that they led to the establishment of the Tuberculosis Research Unit with Sir Geoffrey Marshall as the chair. Professor Waksman and his team went on to discover a range of antibiotics, including actinomycin, clavacin, grisein, neomycin and candidin. Neomycin had widespread uses in creams and lotions. Waksman and Schatz eventually went to court over priority and eventually agreed jointly to be recognized as the discoverers of this remarkable new antibiotic, though Waksman alone became a Nobel laureate for the discovery in 1952.

Back in July 1941, Dr. A. Flynn, editor of *Biological Abstracts*, had posed a question: What should we call these new medicines, derived not from industrial factories but from living fungi? Waksman thought about it at length, and in 1947 he wrote a paper titled, “What is an antibiotic or antibiotic substance?” These terms had been used “rather loosely,” he said; perhaps they should now be “restricted to a specific application.” The medical world had already been using the terms colloquially, and now it was official. Interest in the search for antibiotics was expanding fast. Benjamin Minge Duggar, a botanist at the U.S. Department of Agriculture, discovered tetracycline in 1945, and attention next turned to a fungus with an unlikely origin: It was found in sewage on the Mediterranean island of Sardinia. The fungus was *Cephalosporium acremonium*, which was discovered in 1948 by Dr. Giuseppe Brotzu, professor of hygiene in the medical faculty at the University of Cagliari, Italy. Extracts of this fungus inhibited the growth of *Staphylococcus aureus*, and it could also be used against typhoid. The antibiotic in the fungus turned out to be cephalosporin. Further research was done in London by E.P. Abraham and Guy Newton, who isolated three varieties: cephalosporin P, N and C. Rather than being taken as a tablet, the cephalosporins are best used in ointments and lotions, just like neomycin.

## NEW DISCOVERIES

By this time every major laboratory seemed to be searching for antibiotics. In 1949, a Filipino bacteriologist working for the Eli Lilly Company, Dr. Abelardo B. Aguilar, collected dirt samples for Dr. J.M. McGuire, who isolated a fungus he named *Streptomyces erythreus* and extracted erythromycin from its growth medium. It was launched in 1952 and proved to be successful, though unstable in an acid medium. Japanese pharmacologists at Taisho Pharmaceutical developed clarithromycin (which showed greater stability) from the original erythromycin molecule.

This was followed by the discovery of vancomycin in 1953 from fungi in soil samples brought back by a missionary from Borneo, and by the time gentamycin was discovered in 1963 there was a rapidly growing range of antibiotics. Many scientists believed that the era of infectious bacteria was at an end; I was taught this at university in 1960. By this time, the Beecham Company near London was discovering how to modify the structure of penicillin and soon began to produce semi-synthetic antibiotics, including methicillin, amoxicillin and ampicillin. The battle seemed to be won, and in 1969, the U.S. Surgeon General, Dr. William Stewart, reportedly informed Congress it was “time to close the books on infectious diseases.” I have never located the source of this quotation, though it was a widespread belief throughout the 1970s that antibiotics had conquered the scourge and bacteria had at last been banished.

Methicillin was released in Britain in 1960 to treat infections with staphs that were resistant to penicillin. Because methicillin is a modified molecule, nothing like it existed in nature, and it was believed that bacteria would never become resistant. This was wrong; the first MRSA was detected within two years. Over the next decade, occasional episodes of infection were recorded. In 1974, they caused 2% of hospital infections; within 10 years that had risen to 25%, and just two years later it topped 50%. Now MRSA is everywhere.

For decades, antibiotics were seen as so powerful that they were used in small amounts, allowing resistant organisms to be selected, though Fleming had warned against this hazard in his acceptance speech for the Nobel Prize in 1945. *The New York Times* that year quoted his words: “The greatest possibility of evil in self-medication is the use of too small doses so that instead of clearing up infection the microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out, which can be passed to other individuals and from them to others until they reach

someone who gets a septicemia or pneumonia which penicillin cannot save.” Large amounts of antibiotics are still sold on the open market in countries like Mexico and India, in defiance of Fleming’s warning from 70 years ago. Antibiotics are grossly overused to boost agricultural productivity — in the U.S. about 80 percent of all antibiotics are given to animals, rather than used for treating humans. And all the time that we have been finding new uses for antibiotics, the supply of new ones has been drying up.

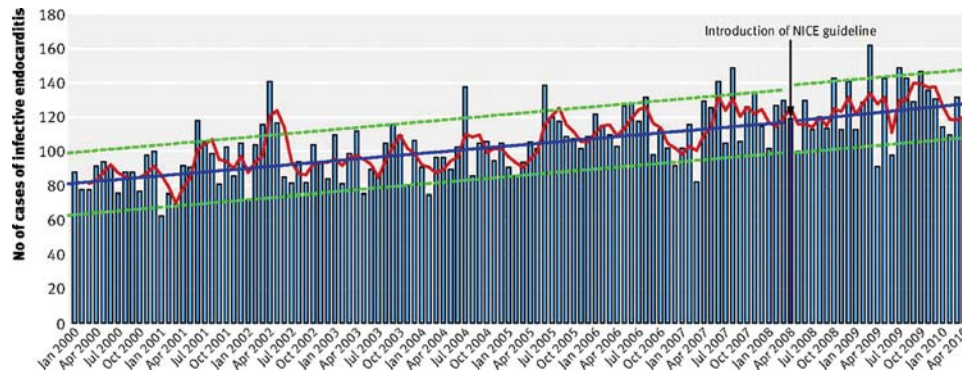
Since the 1970s, the rules and regulations on the development of new drugs have been steadily tightened. Safety testing, compliance, and the new emphasis on medical ethics have made it increasingly difficult to deliver new drugs. The over-confidence of medicine and the overbearing restrictions of the regulators means that for decades new antibiotics have been a low priority. The U.S. Federal Drug Administration (FDA) approved 225 new drugs between 1998 and 2003, of which only seven were antibacterial agents. None was approved in 2002 and, although gemifloxacin and daptomycin were passed in 2003, both were discoveries from earlier years that were approved retrospectively. Currently, there are only one or two under investigation.

Meanwhile, the drug companies are concentrating on other fields of endeavor because what they prefer are drugs that wealthy people take every day, like statins and antacids. In Australia in 1982, Dr. Barry Marshall and Dr. Robin Warren showed that most gastric ulcers were caused not by over-production of acid resulting from stress but by an undetected infection due to *Helicobacter pylori* bacteria. There is treatment available. “Triple therapy” involving omeprazole plus amoxicillin and clarithromycin can conclusively cure the condition. You hear little about this from the drug companies, because they are not so interested in curing the condition but in treating its continuing effects. As a result, few patients are ever given the “triple therapy” — people suffering from these conditions are offered antacids. Sales of drugs like Prevacid, Zantac, Zegerid and that old standby Tums continue to rise. Prilosec OTC alone sells more than \$150 million annually. Antacids sales top \$10 billion every year; and that makes far more economic sense to the pharmaceutical industry than curing the condition in a week.

## A GROWING RESISTANCE

Bacteria, meanwhile, have had a field day. Antibiotic resistance is seen as medicine’s No. 1 preoccupation, and the press are becoming aware of this new





The American Heart Association concluded that using prophylactic antibiotics against bacterial endocarditis was ineffective; in 2008, these antibiotics were widely discontinued. Since then, levels of bacterial endocarditis have certainly increased. However, analysis by the National Institute of Clinical Excellence in Britain shows that rates of incidence had been increasing for many years.

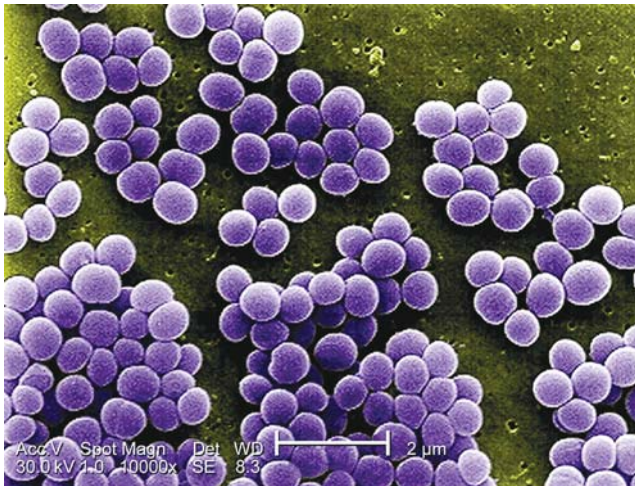
scourge. But bacterial resistance is not a new phenomenon, in spite of what the headlines would have people believe. It is a problem about which we've known for 75 years. In 1940, Dr. D. Gardner, reader in bacteriology at the Dunn School in Oxford, showed that bacteria initially sensitive to penicillin could rapidly acquire a resistance to it. New strains of bacteria that were unaffected by penicillin soon began to appear. The first paper to detail the phenomenon was published by Sir Edward Abraham and Sir Ernst Chain as "An enzyme from bacteria able to destroy penicillin," published in *Nature* in December 1940. At the time, penicillin had not even been introduced into medical practice; so we have known of resistance longer than we have used antibiotics. Dubos warned of resistance in his 1941 essay; it is nothing new. We should have been prepared.

The dawning realization of the magnitude of this problem has caused doctors in the western world to cut back on the amount of antibiotics they prescribe. Let us look at one such change in policy — trying to reduce infections of the heart, bacterial endocarditis. This uncommon disease affects about one person in 20,000 and is caused when bacteria (typically *Streptococcus* or *Staphylococcus*) infect the endocardium that lines the chambers of the heart. The body responds by laying siege and small aggregates of fibrin and blood cells soon surround the invading bacteria. This hinders treatment, for the bacteria are protected against antibiotic therapy by the vegetative mass around them. The people most at risk are those with a pre-existing heart valve abnormality, and it is usually around the valves that these accumulations occur. For decades it was believed that antibiotics should be given to patients prophylactically, but there has now been widespread agreement that this routine administration of antibiotics should cease. A study by the British Society for Antimicrobial Chemotherapy in 2006 first

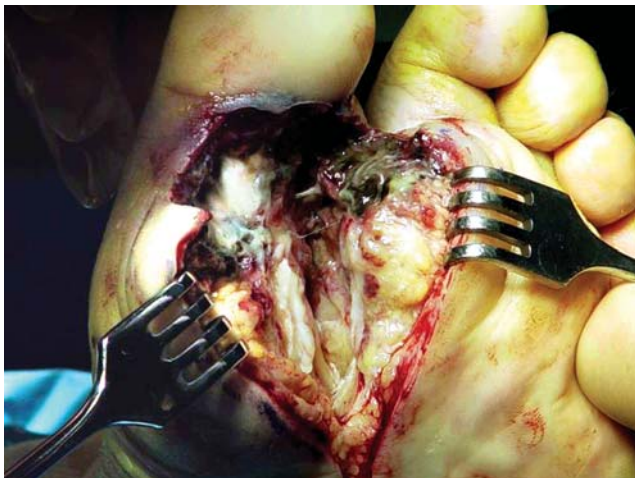
raised doubts about the routine administration of antibiotics, and the American Heart Association reached the same conclusion in 2007. Since 2008, this prophylactic approach has been widely abandoned — while the critics point out that since that date the prevalence of bacterial endocarditis has risen.

This is true. The statistics do indeed show a slow but steady upward trend. Many people have called for the antibiotics to be reintroduced, and some authorities still insist that they should always be administered. However, if we look at the trend in the years preceding the ban, a different pattern emerges. The truth is that the levels of infection have risen steadily in recent decades, and the trend doesn't seem to have been influenced by the change in policy. Here is a case when the cutback in the administration of antibiotics seems to be rational. The use of the drugs was not providing the safeguards against bacterial endocarditis that everyone assumed.

Other changes in rationale are leading to a potential increase in rates of infection. The trend away from using antibiotics for trivial infections means that physicians in the western world are now less willing to treat sore throats with antibiotics. Cases of scarlet fever (once called scarlatina) have begun to increase in frequency, and this is a potential trigger for endocarditis. Scarlet fever is caused by that familiar pathogen *Streptococcus pyogenes* — but not the strep with which we are familiar. The type of bacterium responsible carries a gene that codes for an exotoxin, and the gene is conferred upon the bacterium when it has itself been infected by bacteriophage T12. The earliest symptom of scarlet fever is a sore throat, and prompt antibiotic therapy can prevent scarlet fever from developing. Once it takes hold, damage to the kidneys and heart can ensue, and this previously rare condition is increasing in incidence. In Britain, there were about 1,800 cases per year on average but, since antibiotics have



Methicillin-resistant *Staphylococcus aureus* (MRSA) as imaged by scientists at the Centers for Disease Control and Prevention (CDC) and shown here in false colors. The genus is named from its resemblance to a bunch of grapes (*staphyle* in Greek). A generation ago, MRSA was rare. Today it is infecting patients in virtually every hospital.



Mark Kosinski from the Department of Medicine at the New York College of Podiatric Medicine shows that ceftobiprole can cure infections such as this invasive damage to the foot caused by MRSA. Research into such new antibiotics is not currently keeping pace with the emergence of newly resistant organisms.

been less widely recommended, as many cases have been recorded in a single month. On Jan. 6, 2014, the CDC issued a statement on scarlet fever, reassuring readers that the disease was still “not as common as it was 100 years ago.” I should hope not. In 2011, the authorities in Hong Kong identified a new strain of

strep that is resistant to erythromycin, the drug of choice for the condition; it killed two patients. Today the majority of the streps that cause scarlet fever in Hong Kong belong to this new resistant strain.

In 2008, a resistant strain of *Klebsiella pneumoniae* was identified in Sweden in an Indian patient. The organism produces an enzyme that was identified in 2010 as New Delhi metallo- $\beta$ -lactamase that can destroy antibiotics. This is a new threat, and the gene has since been transferred to other bacteria (including the ubiquitous *E. coli*) and already occurs in India and Pakistan, the U.K., U.S. and Canada and also in Japan. Such outbreaks were, until recently, matters of academic interest — but now they are spilling out across everyday medicine and people are dying because of it. Ten years ago, few bacteriologists had heard of *Clostridium difficile*. It first appeared in drug-resistant form as recently as 2003 and already it is in every hospital.

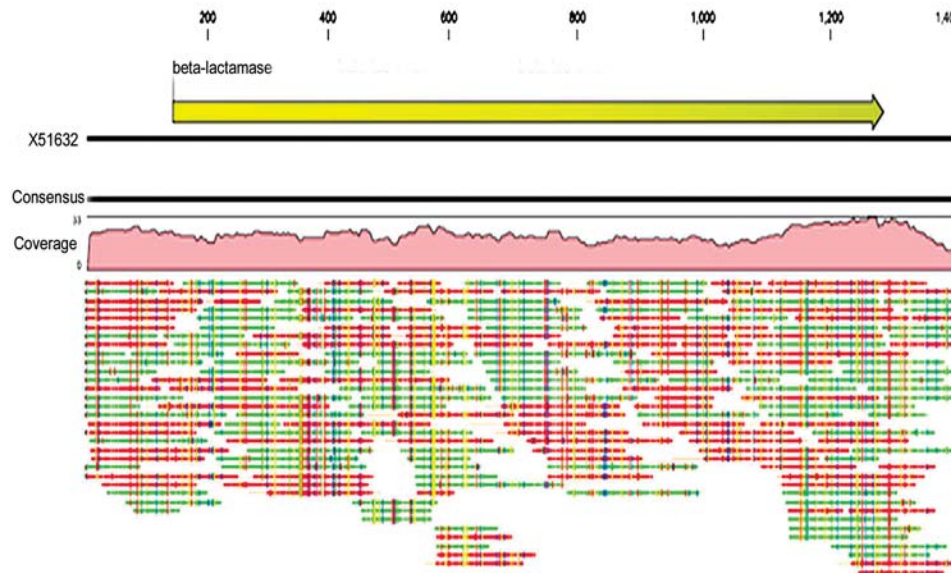
Writing in the *New Yorker*, James Surowiecko argues that the main pressure against companies releasing new antibiotics is that they would have limited application (in case resistance appeared) so they could never turn a profit for the manufacturer. A pharmaceutical company can expect to spend \$350 million to bring a new drug to market. Bearing in mind that many fail after release, the new cost per successful new introduction is about \$5 billion for every successful new drug.

Computer modeling is offering possible new answers. In March 2014, researchers at the University of Notre Dame, IN, announced that they had devised antibiotics that could act against MRSA. The investigators, Mayland Chang and Shahriar Mobashery, believe that their discovery offers a novel approach to inhibiting the growth of bacteria. These are oxadiazole antibiotics, which have proved to be effective in mice. What’s more, they can be taken orally.

## IS OVERUSE THE ISSUE?

It is generally accepted that the overuse of antibiotics in medicine causes this problem. I do not agree. First, resistant strains have been known about since before antibiotics were ever released for use. Secondly, bacteria generally acquire new genes coding for antibiotic resistance through conjugation — they exchange genes through sex. Thirdly, most new threats have arisen from states where antibiotics are less understood and less available than they are in the western world. Obviously, the casual administration of tons of antibiotics to farm animals to increase body growth is not what they were meant for and is not consistent





Before administering antibiotics, it is crucial to know that they work. Gene sequencing could allow us to identify resistant strains of bacteria. The genome of *Citrobacter* has been sequenced by Wendy Smith and Anil Wipat of the Center for Bacterial Cell Biology at Newcastle University and reveals the presence of an enzyme in this bacterium that destroys  $\beta$ -lactam antibiotics like penicillin.

with the wise use of these potent drugs. But overuse in medicine? That does not seem rational.

It is under-use, not overuse, that seems to me to cause the problem. Doctors traditionally gave long courses of high doses, but in recent decades that has changed. Current practice in Britain is to administer antibiotics like amoxicillin without any knowledge of which organism is involved, in doses of 250 mg instead of 500 mg, and to prescribe a five-day course instead of a week. This is dangerous, for it increases the chances for resistant strains to emerge. It has always been unwise to administer antibiotics without knowing whether we are prescribing the best choice; the organism needs to be identified and its sensitivity proved. It would be better to administer double the dose for twice as long; the principle should be to eliminate the pathogen as thoroughly as we can.

We are approaching a catastrophic predicament. Already in the U.S., more than 2 million people will be infected with an antibiotic-resistant bacterium this year (250,000 of them with *Clostridium difficile*) and 15,000 will die. The cost of healthcare alone is \$20 billion, and the knock-on cost to the economy could be double that figure. Every year these figures are going to rise faster. Currently, it is estimated that about half of all antibiotic prescriptions are inappropriate. We hand them out without knowing whether they will work; and yet — in equally worthwhile cases — we simply don't prescribe them at all.

What should we do? First, we must identify whether a bacterium is susceptible to an antibiotic

before treatment begins. Culture and sensitivity testing should be the first step. Secondly, we must administer a high dose to incapacitate the microbe, and thirdly, the course must last long enough to ensure that the pathogen is eliminated from the body. The genomes of many organisms now have been sequenced and we can recognize antibiotic-resistance genes; this needs to be done quickly in future. We could also seek to introduce oligonucleotide tests and use fluorescence labeling to identify the strain. We must find ways to decrease the turnaround time of conventional sensitivity testing. The principle must be to know which antibiotics will kill our pathogen so that we can hit it hard and eliminate it from the body.

We need new antibiotics. Molecular modeling can help us identify new drugs. We need a better business model for research — crude market forces do not provide the stimulus we seek. Phage viruses can kill pathogens but are difficult to deliver to the site of an infection; however, the enzymes they produce (endolysins) can be harnessed. One, embarrassingly dubbed “Staphpekt,” has recently been tested successfully against MRSA in Kennemerland, Netherlands. Bacteria will find it much harder to develop resistance to agents like this.

Rather than overuse causing the problem, it is the opportunism of the bacterial world that our casual attitudes have served only to make worse. Penicillin saved millions of lives for decades but its end is in sight and, until we adopt measures like these, there will be nothing to take its place.