



THE BIRTH OF ANTIBIOTICS

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Did you know that Alexander Fleming was not the only researcher involved in the Nobel Prize-winning discovery of penicillin? Or that it took 13 years for penicillin to be of use for humans? We present an interactive resource to unearth unsung surprises in the process of discovery of penicillin.

School textbooks devote limited space to 'the history of science', often reducing it to dates, names of scientists, a couple of photographs, and a few lines. Students miss out on the exciting 'process of science' and the *eureka* moments that led to those discoveries. Connecting experimental ideas with stories of discovery can offer students an introduction to the nature of science. Not only do students discover how to develop and test a hypothesis, they learn to appreciate the importance of careful observation, patience, and collaborative work in translating discoveries in the lab into applications in the real world. These connections also offer students the opportunity to recognize the time, effort, and rigor involved in serendipitous discoveries.

We explore one such activity-based approach around Alexander Fleming's discovery of penicillin (see **Box 1**). Based on the Vigyan Pratibha learning unit – 'The Accidental Discovery', this approach connects an experiment (**Activity Sheet I**) with an interactive story (**Activity Sheet II**).

Box 1. Curricular links:

This resource is suitable for grade IX and above. It is linked to:

1. Chapter 2 in NCERT's Grade VIII textbook – Microorganisms: Friend and Foe
2. Chapter 13 of NCERT's Grade IX textbook – why do we fall ill?

Activity I: Become a microbiologist!

Encourage students to engage with this activity after they have been introduced to the idea that not all microorganisms are harmful. Lactobacilli in curd and yeast in bread can be offered as common examples of microorganisms that are, in fact, beneficial to humans.

Can we demonstrate the presence of microbes in curd without the aid of a microscope? Surprisingly, one doesn't need to be a trained microbiologist to do this. This activity (see **Activity Sheet I**) allows students to grow microbes from curd (~bacterial inoculum/seed) on a cooked potato-slice (~solid substrate), thereby avoiding difficult techniques of classical microbiology (sterilization, media preparation, specific apparatus, and aseptic conditions). To do this, some curd is evenly spread out on a cooked potato slice, and incubated for 24–48 h. By the end of this period, students can see many small whitish dots on the slice – each of which is a *Lactobacillus* colony (see **Fig. 1a**). Each **colony** consists of several million bacteria growing in a single pile that is visible to the naked eye. Students may also observe that the colonies are quite diverse in terms of their size (smaller, bigger), appearance (some translucent, others opaque) and so on.

To observe the effects of antibiotics on microorganisms, some of the potato at

the center of each slice is scooped out to create a small, shallow well. Antibiotic solutions of different concentrations are added to different wells. Potato slices with only curd or only antibiotic can serve as parallel controls. By the end of the incubation period, students will be able to observe the presence of a clear zone of inhibition around a potato well with antibiotic solution. In other words, bacterial colonies will be fewer in number or absent from this zone (see **Fig. 1b**). Students may also observe that the size of the clearance zone increases with an increase in the concentration of antibiotic added to the well. Other factors that could lead to variations in zone size include handling of the slices, and errors while cutting, pipetting, and seeding.

Through this activity, students will not only observe what microbial growth looks like, but will also appreciate the visual concept of a microbial colony. Since these colonies appear only on potato slices with curd, students will be in a position to infer that they belong to the curd bacteria. Observing a clear zone of restriction around the antibiotic wells will give students visual proof of the inhibition of bacterial growth by a diffusing antibiotic (see **Box 2**). It will also help solidify the idea of what Fleming is likely to have observed around the fungal colony that produced penicillin.

Box 2. Extended activity:

Students can also use this experiment to check –

1. The presence of microorganisms in soil – by seeding the potato slices with a soil sample instead of curd, or
2. Differences in the nature of microbial inhibition – by using different sets of antibiotics.

Activity II: Retracing the accidental discovery of penicillin

This interactive story-based activity (see **Activity Sheet II**) describes the discovery of modern penicillin through questions that encourage 'critical thinking'.

The story introduces students to the process of science, while the questions

offer avenues for open thought and discussion. To ensure maximum participation by students, we highly recommend that teachers:

- Avoid offering direct answers, and encourage discussion instead.

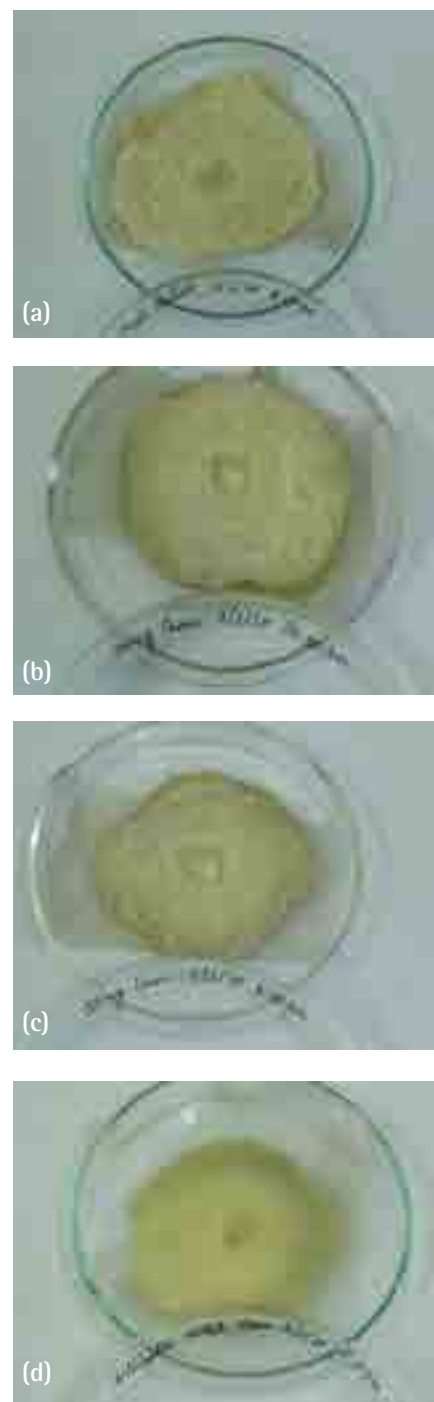


Fig. 1. Effect of Chloramphenicol (Cam) on potato slices spread with curd. Plates with – (a) curd control, (b) 20 mg Cam, (c) 50 mg Cam, and (d) antibiotic control (20 mg Cam). Fewer colonies are observed around the wells in the slices with 20 and 50 mg Cam. Clearance is seen on one side of the well in the slice with 20 mg Cam, and around the well for slice with 50 mg Cam. This clear zone indicates the inhibition of lactobacilli by the antibiotic, which diffuses into the slice along a concentration gradient (with the highest concentration at the center).

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Box 3. Teacher's box:

1. Teachers may need to introduce the idea that mould are fungi whose spores are ubiquitously present in the air around us. Most saprophytic fungi grow at moderate room temperatures (e.g., mushrooms at 25°C), while disease causing bacteria tend to grow better at 37°C (human body temperature).
2. A debate around who should've received the Nobel Prize for the discovery of penicillin can be an interesting exercise for students. Although the prize was awarded to Alexander Fleming, Howard Florey, and Ernst Chain, students may suggest that Heatley and the 'Penicillin girls' were deserving, yet unsung, heroes in this process.
3. The world wars reduced funds for research, forcing many scientists to limit their resources. But scientists involved in the discovery of penicillin were driven by the need to produce enough antibiotic to save soldiers wounded in war. Students may, therefore, conclude that in a way, the war pushed efforts to produce penicillin in larger amounts. This discussion is likely to help students explore the impact that politics can have on scientific discoveries.
4. Teachers may need to provide clarity regarding the role of antibiotics. Penicillin doesn't heal wounds. During an infection, bacteria multiply to very large numbers, which overwhelms the immune system. Penicillin slows down bacterial growth, indirectly giving the body's immune system a better chance at fighting the infection.

- Consider all student responses without classifying them as right or wrong. Many questions need not have one right answer, and not being too critical or disapproving of students' views will encourage open thinking.
- List all student responses/ideas on the board and moderate a student debate around them. This will allow students to engage with each idea, and reflect on their reasons for agreement/disagreement.

Discussion around this activity can take many directions (see **Box 3**). Consider one question in the activity – what in the bread mould helps wounds heal? Student discussion around this question can be followed up with another question – is it possible that the mould on bread releases some chemicals that could kill or inhibit the growth of bacteria in wounds? Once students have explored this possibility, teachers could present the fact that bread acts as a nutrient medium for all kinds of moulds to grow. Even if ancient Egyptians knew of 'ways' to help a wound heal better, they would have less control over what kind of moulds grew on bread. What if some of the fungi or mould on the bread piece used for healing were

non-beneficial or even toxic? Discussion around this part of the activity can help students differentiate between antiseptics used in traditional medicine from antibiotics in modern medicine. This could be used as an example of how the identification of the active component involved in the Egyptian healing technique by modern scientific principles allows us to minimize mishaps from use of ineffective or toxic moulds. It could also be used to illustrate how large-scale production and purification technologies have made penicillin available for use at the most effective clinical dose.

Before winding up, teachers could use some of the themes provided in parentheses to encourage students to think about the following open-ended questions:

- What **new** things did you learn from this exercise? (Themes: effect of chance, accidental discoveries, collaborations, working in teams).
- How is learning about the 'history of antibiotics' significant now? (Themes: drug discoveries today also have to go through similar time-consuming and rigorous procedures of animal and clinical trials before their benefits can reach patients).

- Bacteria and fungi have existed throughout human history. What was it that triggered the discovery of penicillin in 1928? (Theme: the accidental observation of bacteria and fungi growing on the same plate, uncovering their antagonism).
- If there were so many scientists and assistants involved in the discovery of penicillin, why is only Fleming's name popularly associated with it? (Theme: primary discoverer).

Parting thoughts

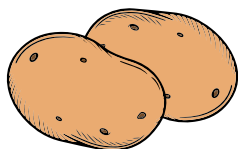
Students are never really offered an experience of the scientific process. How do ideas nucleate from observations of the natural world? How do we use the scientific principle of controls and rational thinking to find scientific explanations for observed phenomena? These activities aim to offer students this experience by engaging with both narrative and experimental evidence. They also provide an introduction to the world of microbes; helping students appreciate how some microbes are useful, while others are harmful. Finally, it helps students understand that the fruition of any idea in science involves many people, although only a fortunate few get recognized or awarded the Nobel Prize for their contributions.

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ACTIVITY SHEET I: BECOMING A MICROBIOLOGIST!

You will need (per group of 3-4 students):

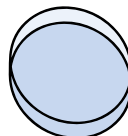
2 medium-sized potatoes



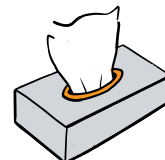
2-3 spoons curd



4 Petri-dishes or watch glasses



Tissue paper

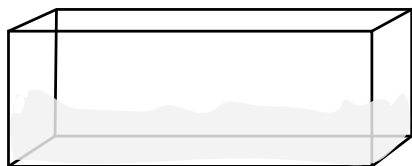
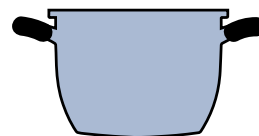


Amoxicillin or any broad-spectrum antibiotic (obtained from discarded medicines or a pharmacy) dissolved in water at a concentration of 100 mg/ml

A dropper or glass pipette (with graduated markings)



Boiled water (cooled to room temperature)



A large plastic box lined with tissues wet with a slight excess of boiled water to create closed moist chamber

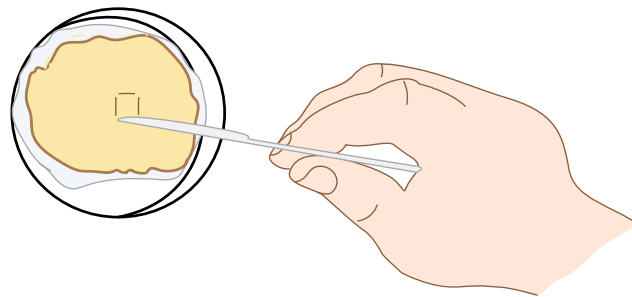
What to do:

1. Boil two medium-sized potatoes for ~20 min, and allow them to cool.
2. Clean a Petri-dish or watch glass and place a folded piece of tissue at the base. Keep the tissue moist with the boiled water.
3. Peel one of the potatoes and cut into ~0.5 cm thick oval slices along its length, maintaining uniform thickness.
4. Place a slice on the wet tissue in one watch glass. Do the same for the other three watch glasses.
5. To the centre of three of the slices, add 1 ml of curd/buttermilk each using a dropper or pipette. Use a clean spoon to spread the curd evenly on the slice until it gets completely absorbed. Label these slices as:
 - 'Curd control' or CC,
 - '20 mg', and
 - '50 mg'.
6. Take another slice of potato. Do not add curd to this slice. Label as 'Antibiotic control' or AC.
7. Use a scalpel to scoop out a shallow well in the middle of all four slices, ensuring that the wells do not get punctured at the bottom.
8. Place all four watch glasses in the moist chamber.

9. Add:

- 0.2 ml of antibiotic solution to the well in the slice labelled '20 mg'.
- 0.5 ml of antibiotic to the well in the slice labelled '50 mg' as well as the one labelled AC
- 0.5 ml of pre-boiled water to the well in the slice labelled 'CC'.

10. Without disturbing the plates or watch glasses, close the chamber and keep in a warm place for 24–48 h.



How to cut out a well from a potato slice.

Cautionary handling note:

Wash your hands thoroughly with soap after handling the rotten slices of potato, and do not bring eatables near them. Once the experiment is completed, boil all the used potato slices in a beaker for 10 minutes, strain, and discard as wet waste. Students with a history of prior allergies should take extra precautions.

Record: At the end of 24 h, observe each plate and record your observations in the table below.

Plate	What we see	What we infer from our observations
CC		
AC		
20 mg		
50 mg		

Discuss:

1. Do you observe any difference in the number of colonies that appear around the wells in the slices containing 20 mg and 50 mg antibiotic? If yes, why do you think this happens?
2. How would you use this experiment to find a treatment for infectious bacteria?



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ACTIVITY SHEET II: RETRACING THE BIRTH OF ANTIBIOTICS

Did you know that the first antibiotic was discovered by accident? The fungus (*Penicillium notatum*) that produces penicillin was discovered in 1928, but it was first used in humans only in 1941. Why did such an important discovery take so long to reach the common man? Let's retrace the discovery of penicillin, and understand how it became a life-saving antibiotic. This activity will help you uncover this story using your own thoughts and ideas!

This story is told in many parts, with questions at the end of each part. Try and answer all the questions at the end of one part before moving on to the next part. If you skip the questions at the end of each part, you will miss out on all the fun.

Now, for the first part of the story.

Today, a small cut or wound is not a very serious thing to happen to anyone. Some pain/discomfort can be easily managed, thanks to a range of antiseptic creams, lotions, and powders. But, did you know that before antibiotics became available, people would die of even minor cuts, burns, or injuries? This was because their wounds would get infected by bacteria, causing severe infections, toxic shock, or even death. The lifespan of an average Indian was 32 years, as many suffered fatal infections. Treating such patients was a challenge to hospitals, doctors, and scientists all over the world. In fact, even until about 90 years back, the treatment of wounds was a big challenge.

QUESTION

How would you define an antibiotic?

In ancient India, turmeric was used to treat wounds. However, turmeric is considered as an antiseptic or Ayurvedic drug, and not an antibiotic. How would you define an 'antibiotic'? Here's a clue – one type of microbes produces it against other types of microbes.

Interestingly, although antibiotics were not known to the ancient Egyptians, they would apply a mouldy piece of bread on infected wounds to help them heal better.



Ancient Egyptians would apply a mouldy piece of bread on infected wounds to help them heal.

THINK

What in a mouldy piece of bread could help in the healing of wounds?

In Fleming's shoes

We know that certain bacteria and fungi produce compounds that can kill other microbes. These chemicals help the bacteria and fungi survive in a competitive environment. This discovery was first made in the year 1928 by the scientist Sir Alexander Fleming.

Fleming was studying an infectious bacteria called *Staphylococcus* (let's call it *Staph*) at a hospital in London. *Staph* causes a wide range of infections – from pimples and sore throats to pneumonia and major infections of the urinary tract. Fleming would grow *Staph* cultures in Petri dishes in his lab. One day, Fleming accidentally left a Petri dish near a window. It so happened that he left for vacation the next day.



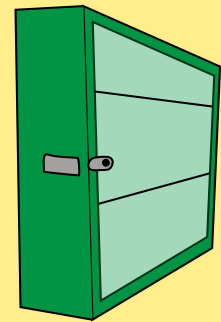
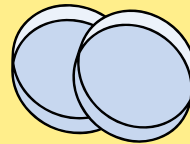
Alexander Fleming is credited for the discovery of antibiotics.

Do you know HOW bacteria are grown in laboratories?

Just like us, bacteria also require food. Scientists provide food in the form of a 'nutrient medium' to bacteria on plates called 'Petri dishes'. Each such dish has a lid which can be closed. Petri dishes with the nutrient medium are kept in a temperature-controlled closed chamber, called an incubator. The temperature in an incubator can be adjusted to best suit the particular bacteria or other microorganisms we intend to grow in them.

Bacteria can be grown in labs with:

Petri dishes.



An incubator.

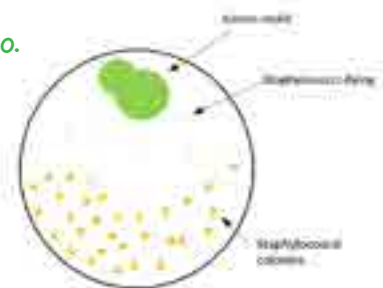
Upon returning from his vacation, Fleming noticed that a contaminating green mould (other than *Staph*) had grown on the plate by the window. Surprisingly, the *Staph* colonies closest to the mould were much smaller in size. Some appeared to be dying, as if the mould had released something in the area around itself!

Can you see a single bacterial cell with a naked eye?

Guess not, right? What if millions of bacterial cells clump together? Well, then you can see them with the naked eye! When a single bacterial cell divides into millions of cells in a pile, this mass becomes visible to the naked eye, and is called a 'colony'. The size of a bacterial colony can vary from that of a pin head to a small button.

THINK

- Q. Fleming was trying to grow bacteria on his plates. But, he ended up getting some mould too.
- How do you think the mould entered the plate?



Green mould on a plate containing Staphylococci.



- Is the mould a fungus or a bacterium?

- Why do you think bacteria were not able to grow near the mould?

Q. The contaminated plate had been left near a window. What if Fleming had remembered to keep the plate inside an incubator (at the optimum temperature for growing *Staph*)? What do you think is likely to have happened then? Would the fungus have grown more slowly (since mould grows on bread left at room temperature)?

Q. Imagine you were Fleming – what would you have done if you saw the plate with the strange unwanted mould?

The contaminated plate got Fleming thinking: why were bacteria unable to grow near the mould? He identified the mould to be *Penicillium notatum*, and wondered if the mould was releasing some antibacterial chemical into the plate. So, he grew the mould in a nutrient-rich liquid. The liquid also showed antibacterial activity. Fleming named the antibacterial chemical that the mould had released into the nutrient media 'penicillin'.

Fleming wondered if penicillin could be used to control bacterial infections in humans. There were two challenges. The antibacterial activity of the culture media in which the mould was grown was short-lived. And, to be useful for humans, the chemical with the antibacterial activity would need to be separated from the rest of the chemicals in the culture media. Over the next 10 years, Fleming tried several different ways to purify an active form of penicillin from the mould's culture media, but remained unsuccessful.



THINK

If you were in Fleming's shoes, how would you have addressed this problem?

The first human use of penicillin

Fleming addressed this problem by handing it over, in 1939, to Howard Florey and Ernst Chain, scientists at Oxford university. The two of them teamed up with another scientist – Norman Heatley. They used methods different from what Fleming had been using, and managed to get an 'active' form of penicillin that same year.

To check how effective this active form of penicillin was against infections, the three scientists infected some laboratory mice with a highly infectious bacterium, and gave only half of them penicillin.

THINK

Q. Why do you think the scientists gave penicillin to only half the infected mice?

Q. What do you think happened to the infected mice which:

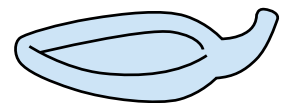
- Received penicillin?
- Did not receive penicillin?

Q. Why do you think the scientists tried the active form of penicillin on mice first?

Hint: Did they know if penicillin was safe for human consumption?

The infected mice that didn't receive penicillin died. Thankfully, the ones that received penicillin survived the infection. This confirmed that the active form of penicillin was not just active and harmless to mice, but also helped control bacterial infection.

The next task was to produce penicillin in sufficient quantities for human use. On the one hand, Florey went on to employ six women, famous as the 'Penicillin women', at £2 per week to 'farm penicillin' from the mould. On the other hand, Heatley used literally every food tin, bed pan, and bottle he could find to grow the mould. He even designed 500 stackable ceramic bedpans for this purpose. In spite of their best efforts, and cultivating the mould in hundreds of litres of culture media, the three scientists were only able to obtain a few milligrams of the active form of penicillin.



Heatley designed 500 stackable ceramic bedpans to grow Staph.

We started this story by describing how this was a time in history when even minor injuries caused death. One example of this came in 1941, when a man called Albert bruised his mouth with rose thorns. The bruises slowly developed into a life-threatening infection. Albert was given penicillin and

showed some signs of recovery. But, he died soon after because there wasn't enough penicillin to cure him completely. This shows how badly penicillin was needed at the time – even for minor wounds.

The wonder drug

Research in British universities was badly hit by the Second World War. Florey and Heatley moved to the U.S. to continue with their efforts on producing sufficient amounts of active penicillin. It was here that they made an unexpected discovery. An assistant brought along a fruit with a 'golden mould' growing on it. It turned out that this mould (*Penicillium chrysogenum*) was a close relative of *Penicillium notatum* (the green mould observed by Fleming). To their delight, Florey and Heatley discovered that the golden mould yielded 200 times more penicillin than Fleming's green mould!



THINK

Can you guess the name of the fruit with the golden mould?

(Hint: It is a summer fruit)

M _ _ K _ _ L _ N

Eventually, these scientists succeeded in producing active penicillin in large amounts. The antibiotic saved many hundreds of soldiers injured during the war from death by bacterial infection. This wonder drug has since become one of the most important discoveries of the millennium!



THINK

Q. In what ways do you think the war may have affected the process of discovery of penicillin?

Q. How do you think penicillin helped save millions of lives during the war? Does it help in the healing of wounds?

Q. Imagine that you are part of the committee deciding who should be awarded the Nobel Prize for the discovery of penicillin. You can only choose three people to award this prize to. Now that you have read the entire story, which people would you choose? Why?



Q. Connect the words here using arrows. Write an appropriate linking phrase above each arrow. You may choose linking phrases from these – ‘if left’, ‘may lead to’, ‘could result in’, ‘if treated with’. Or, you could choose any other phrase you find appropriate. The first one is done for you.

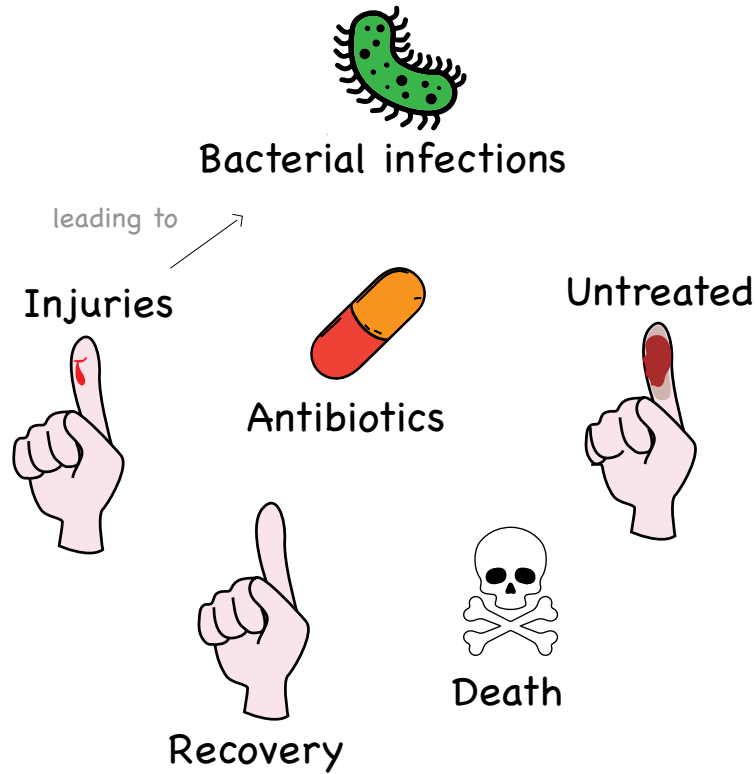


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Key takeaways

- Re-tracing Fleming's chance discovery of penicillin will offer students an appreciation for the importance of keen observation in science, and the role it plays in serendipitous discoveries.
- An understanding of the struggle to generate penicillin in amounts required for human use will help students appreciate that a chance observation is only the beginning of a complex (scientific) process that demands time, patience, and rigor.
- Recognizing the contributions of Howard Florey, Ernst Chain, Norman Heatley, and the 'Penicillin girls' in bringing penicillin from the bench to the bedside will help introduce students to the highly collaborative nature of modern science.
- Reflection on the role of war, and the unsung contributions of many people involved in the story of penicillin will offer students a better understanding of the political and human dimensions of science.
- By actually growing microorganisms in the presence of antibiotics, students will be able to appreciate Fleming's initial observation and learn a new way to query the world of microbes around them using simple kitchen equipment.



Acknowledgements:

The authors would like to thank Leena Phadke, Associate Professor (Retd.) Ramnarain Ruia College, Mumbai, for her ideas on the potato slice experiment, and development of the narrative on the discovery of penicillin. We acknowledge the support of the Govt. of India, Department of Atomic Energy, under the Vigyan Pratibha Project (No. R&D-TFR-0650). We also thank the DBT Ramalingaswami Fellowship, all the students who were part of the field trials for these activities, and all Vigyan Pratibha members for providing their inputs during the development of these activities.

Notes:

1. This article is based on the learning unit 'The Accidental Discovery' from the Vigyan Pratibha project (<https://vigyanpratibha.in/index.php/the-accidental-discovery/>), currently undertaken by the Homi Bhabha Centre for Science Education, TIFR, Mumbai. Vigyan Pratibha is a central government initiative for students of grades VIII-X of Kendriya Vidyalayas, Jawahar Navodaya Vidyalayas, and Atomic Energy Central Schools. This science nurture programme aims to develop critical thinking skills among students of diverse backgrounds by engaging them in an activity-based approach to learning school science and mathematics.
2. Source for the image used in the background of the article title: https://commons.wikimedia.org/wiki/File:Three_tubes_of_penicillin_powder,_two_of_International_Stand_Wellcome_L0059014.jpg. Credits: Wellcome Images (<https://wellcomeimages.org/indexplus/image/L0059014.html>), a website operated by Wellcome Trust, Wikimedia Commons. License: CC-BY.

References:

1. Douglas Allchin (2002) Scientific Myth-Conceptions, Fourth International Seminar on the History of Science and Science Education: Issues and Trends, Stephen Norris (Section Ed.) URL: <http://douglasallchin.net/papers/myth.pdf>.
2. Alexander Fleming biography: <https://www.biography.com/people/alexander-fleming>. Last updated: Jun 26, 2019.
3. Discovery and Development of Penicillin. International Historic Chemical Landmark. URL: <https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html>.
4. Howard Markel. The Real Story Behind Penicillin. PBS Newshour. URL: <https://www.pbs.org/newshour/health/the-real-story-behind-the-worlds-first-antibiotic>.
5. Alexander Fleming (1929). On the bacterial action of a culture of *Penicillium* with special reference to their use in the isolation of *B. influenzae*. *British Journal of Experimental Pathology*, 10(3): 226–236.
6. Kelly Swain (2017). The Penicillin girls (and guys). *The Lancet*, Vol 389, pp.1507.



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