The Accidental Discovery

Overview

This unit is based on History of Science and involves two tasks. In task 1, the students will try to grow their own microbes from soil on a potato slice. The novelty of observing the diversity of microbes around them will provide a good learning opportunity. This will also enable them to demonstrate the ubiquitous nature of microorganisms. In task 2, students will figure out how the ubiquitous nature of microorganisms led to one of the most popular discoveries of the millennium- antibiotics! In this task, students will be unfolding a major scientific discovery in the form of a story followed by questions for critical thinking. The task will enable them to understand the importance of observation, analysis, collaboration, patience while conducting scientific experiments. Students will be able to understand that discoveries involve years of hard work and are affected by various social and political factors.

Time required:

4 sessions of 40 min each (2 sessions for each task)

Type of learning unit

Task 1: outdoors and laboratory, task 2: classroom

Introduction

Microorganisms are ubiquitous in nature and are present in air, water and from deep seas to hydrothermal vents. They are also present on and inside the human body. Microorganisms are uniquely adapted to their environment for survival. Many types of microorganisms grow together in the same habitat. They can help each other grow together, or can be harmless to each other. Some types of organisms compete with other types in case there is a competition for nutrients. They may produce ‘antibiotics’ which can kill the competing microorganisms.

The first antibiotic to be discovered was penicillin from the mold *Penicillium notatum* . After the discovery of penicillin and its tremendous success in preventing bacterial infections in wounded soldiers in World War II, newly discovered antibiotics became first line of treatment to control bacterial infections.

We all are aware that micro-organisms are present everywhere around us. In task 1, we present the students a tool to ‘grow bacteria’ from their garden soil or from waste water on simple medium like cooked potato slices. Students will be able to demomstrate the diversity of microorganisms in soil, water and air.
It was a fungal spore present in air which accidentally entered a plate on which bacteria were being grown. This fungus was *Penicillium notatum* which produced the antibiotic penicillin. Although penicillin was discovered in 1928, it was administered in humans only in 1939. Why did a discovery take so long to reach the humans? Did you know that there

were some more scientists other than Alexander Fleming involved in the discovery of penicillin?

Discoveries are often given a limited space in school textbooks and the information is restricted to the name of the scientist, a photo and few lines on the discovery. Although the assessment does not always include the part on discoveries, students might miss out on a very interesting account of ‘science in the making’. If the discoveries are read in the form of a story, as given in task 2, students would understand the aspects of nature of science, the significance of scientific processes like observation, hypothesizing, collaboration, efforts and patience that go into scientific research. More importantly, students would also realize that discoveries may happen overnight but appropriate use of the discovery may take much longer.

Learning objectives:

After working on this learning unit students will understand that:

a) Scientific discoveries can be accidental.
b) Scientific process is complex, requires time, patience and team-work.
c) A particular research study does not end with discovery, rather, it results in further experimentation.
d) Observation and interpretation are important aspects of research.
e) Antibiotics are naturally produced by some microorganisms present in the nature.

Pre-requisites for the unit

Before working on the tasks, students must be aware that micro-organisms are present around us. They should know the difference between fungi and bacteria, diseases and infection.

Links with the curriculum

1) Class 8th, Chapter 2 **Microorganisms: Friend and Foe**-Medicinal use of Microorganisms, small panel on discovery of antibiotics
2) Class 9th, Chapter 13 **Why Do We Fall Ill?** How antibiotics function against bacteria, Why antibiotics do not act against viruses, activity on use of antibiotics, principles of treatment

References and suggested readings

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.
2. Alexander Fleming biography https://www.biography.com/people/alexander-fleming-9296894
3. Discovery and Development of Penicillin. International Historic Chemical Landmark. //www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html
4. Howard Markel The Real Story Behind Penicillin PBS Newshour 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 *Penicilium* with special reference to their use in the isolation of *B. Influenzae*

Task 1. Crowded plate technique-using fertile soil or waste-water

- In this task, students will try to grow bacteria on a potato slice. The source of bacteria could be fertile soil or waste water.
- For this task, students would again work in groups of 3-4 persons. Encourage different groups to get soil or waste-water samples from different locations.
- This task requires a cooked medium sized potato, knife and ear buds.

  Instead of ear-buds, cotton wrapped around a match stick could be used. The task to be done in a laboratory in presence of a teacher/facilitator.
- The experiment may require 30-40 min to perform. The slices will have to be kept in the lab for 2-3 days. Everyday, after 24 hours, students can come and observe the slices for any changes.
- Teachers can click pictures of the slices every 24 hours and share them with us!

Do you recollect from previous year's knowledge, that microorganisms are present everywhere in nature? A variety of microorganisms are present in soil, air or water. These microorganisms produce and secrete various chemicals which might affect bacterial growth.

Have you ever tried to 'grow' microorganisms in a lab? Let's try to grow them from soil or water. We will perform a simple experiment using potato, waste-water or soil, cotton buds, clean plate.

Different groups may use soil or waste-water samples collected from different places. Preferably, use fertile soil.

> Divide the class into groups of 4 each. Ask each group to get soil or waste-water sample from near the school/home.

Method:

1. Boil a medium-size potato for 20 min and allow it to cool.
2. Take a spoonful of soil in a clean tube and add 4 ml of boiled water to it. Mix it properly with a glass rod and let it to stand for a few minutes to allow soil particles to settle down.
4. Swab a petridish or watchglass with ethanol and place a moist tissue at the base. You may use boiled and slightly warmed water to moisten the tissue paper. Make sure the water is not very hot.
5. Peel off the potato and cut 0.5 cm thick slices. Thickness of the slice should be uniform all over. Place each slice on the moist tissue paper. Each group gets 3 slices of potato.
6. Dip a cotton bud into the soil suspension / waste-water sample and spread it in all directions on the potato slice. Use different buds for different slices. Label each plate after spreading the sample with the location of soil / source of water.
7. Additionally, on another slice, spread the boiled water using an ear bud. On another slice, do not spread anything. Label these as 'controls' with and without boiled water.

8. Cover each slice with a beaker previously swabbed with ethanol or alternately cover all slices together with a large tray swabbed with ethanol. Ensure that the container is clean. Keep the setup undisturbed for a day.
9. On the next day, observe the slices for any microbial growth. If the slice appears dry, add some boiled and cooled water to moisten the tissue paper beneath.
10. Observe the slices carefully every day up to 2 days and interpret.

Q 1. What all do you see? You may draw a figure with the use of colors, if possible.
Do you see a difference between day 2 and day 3?

Q 2. Why did we take a slice spread with water only and a slice on which we did not spread anything?

Q 3. How will our interpretations be affected if we don't take the slices spread with water only and another slice without spreading anything?

Record the results as per the following table:

Group no.	Sample Soil/water	Source	Observations (type of growth, colour, texture, etc.)

Take a small drop of distilled water on a slide. Add a very small part of the microbial colony to the water drop using a blunt needle. Cover the mount with a cover slip and observe under the microscope.
Take a look at other groups' mounts under the microscope.
What can you say about the diversity of colonies from various groups in your class?
Try to draw the images as you observe under the microscope.

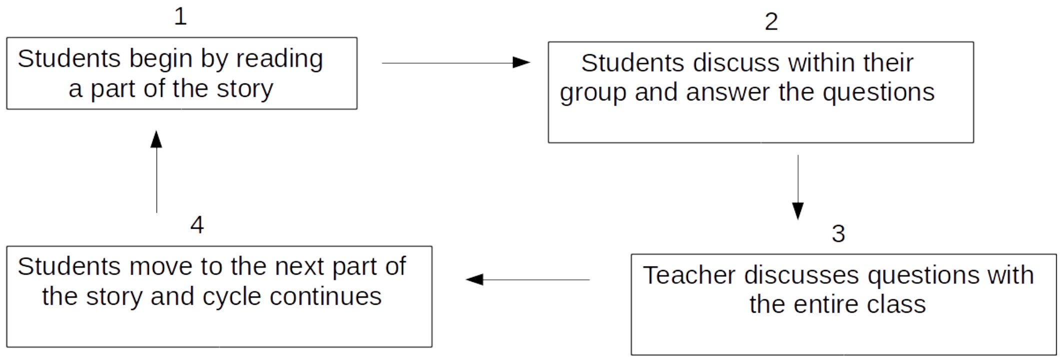
For disposal of the potato slices after the experiment:

- Take a large tray and add a spoonful of liquid soap/detergent powder
- Add water to the tray and work a rich lather
- Put all the potato slices into this tray and leave for 1-2 hours
- Decant the water using a strainer and wrap the slices in a paper bag
- They can be now disposed off as ‘wet waste’.

Task 2: Uncover the story

Begin the session with the following question:

> Teacher: All of us, sometime or the other, get a cut or a wound. Do you know, then, in how many ways, a wound can be treated?
> Students: (Expected answers) wash with water, soap, apply antiseptic, apply band-aid, etc.
> Teachers: Can a small cut or wound lead to death? Why?
> Students may say ‘no’.
> Teacher: What is the worse that can happen if you get bruised by, say, rose thorns? Can it result in death?
> Let students discuss and answer whatever they feel appropriate.
> Teacher: As we go through the task, we will see what all can happen if someone is bruised by rose thorns?
>
> Handover the worksheets to the students
>
> Now, divide the students in groups of 4 each. It is very important that students first read, discuss and answer the questions of one part before going on to read the next part. If they read the next story part without answering the questions, they might miss out on the fun!
>
> Teachers may follow the flowchart as shown below for conducting task 1


1
Students begin by reading a part of the story
2
Students discuss within their group and answer the questions
3
Teacher discusses questions with the entire class
4
Students move to the next part of the story and cycle continues

For discussing with the entire class

- Encourage maximum participation by students during the discussion
- It is strongly recommended not to give the answer directly without any discussion.
- Consider all possible answers/responses, without classifying them as 'right/wrong'.
- The responses may be written on the board and students may themselves debate over the ideas.
- For most of the questions, there need not be one and only one right answer. Welcome all the possible responses for such questions. Other students may be asked to 'agree/disagree' with the responses along with their reasons to agree or disagree.
- It would be helpful not to be to be too critical or disapproving of students' views.

Uncover the story!

Do you know how penicillin was discovered? The fungus *Penicillium notatum* produced the antibiotic penicillin. Although penicillin was discovered in 1928, it was administered in humans only in 1939. Why did a discovery take so long to reach the humans? We present to you a story on the discovery penicillin how it became a life-saving antibiotic. But you will uncover the story using your own thoughts and ideas!

Important: Go sequentially. If you skip the questions and read the story parts first, you will miss out on all the fun. So, try and answer all questions of one part before moving on to the next.

Have you ever seen mold growing on bread? What do you do with such a piece of bread? Ancient Egyptians would apply this moldy bread on infectious wounds to treat them. How would a mold help in healing of wounds?

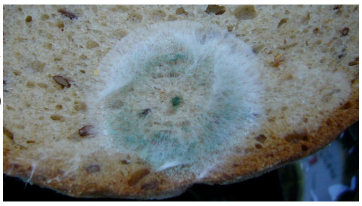


Image source: Wikimedia Commons

Before the discovery of penicillin, minor injuries like cuts or scratches could lead to severe bacterial infections or even to death. Hospitals would be full of people with blood poisoning due to major or minor injuries.

Image source: Wikimedia Commons

In those days, Sir Alexander Fleming was a scientist at a hospital in London. In the year 1928, he was performing an experiment where he was growing cells of a bacteria called **Staphylococci**. For convenience, let's call it Staph!

Image source: Wikimedia Commons

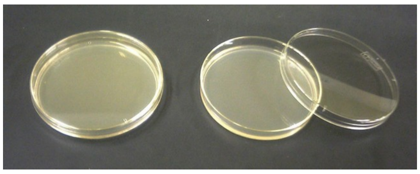
These bacteria can cause a wide range of infections- from minor skin infections to major infections like pneumonia, toxic shock syndrome, or urinary tract infections.

Do you know how bacteria are grown in laboratories?

Just like humans, bacteria also require sources of nutrients- carbon, nitrogen and salts. Bacteria, isolated from soil, water, air skin, teeth or fluids like blood, urine, stool, etc. are grown on a nutritious mixture called **culture medium** which may be liquid or solid. When the medium is solid, it is usually placed in a dish called 'petri-plate'.

The dish has a lid and the bacteria in the plates are allowed to grow in closed plates, kept in a chamber called **incubator**.

The incubator is like a small cupboard which can maintain a set temperature. When bacteria are made to grow in a lab, the incubator is set at a specific temperature most suitable for the bacteria.



Petri-dish

Incubator

Another question, can you see a single bacterial cell with a naked eye?
What if millions of bacterial cells clump together?

Then, you can see them with a naked eye!

These millions of cells together form what is called as a 'bacterial colony'. The size of a colony varies from a pin head to the size of a hole made by the punching machine.

Going back to 1928…

While the Staph were growing in his lab, Fleming went on a vacation and when he returned, he noticed that the plate was negligently left outside, near a window, instead of in the incubator. He observed that something other than Staph had also grown on the plate. Look at the image of the plate below.

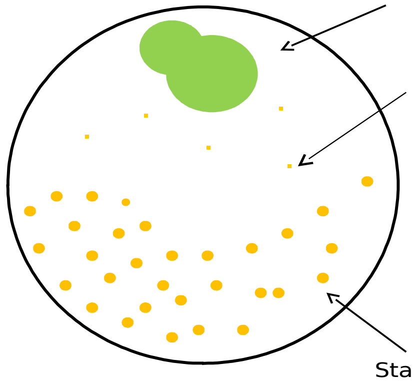


Image adapted from Fleming, 1929

Q. Have you observed this kind of a green mold before? Is mold a fungus or a bacterium? Why?

> Green molds usually appear on fruits or vegetables like gourd or at times on bread.

Q. If Fleming was trying to grow only bacteria, from where do you think the mold entered the plate?

> Students know about the ubiquitous nature of micro-organisms. They know that micro-organisms are present in the air.

Story continues….

If Fleming was trying to grow only bacteria, from where do you think the mold entered the plate? Instead of throwing the contaminated plate straight away, Fleming looked carefully and observed the absence of bacterial colonies closer to the mold. That set him thinking **why the bacteria were not able to grow near the mold**.

Q. Can you help him answer?

> The mold could have produced something which prevented the bacterial growth.

Q. If you were in place of Fleming, how would you make use of the process where bacteria cannot grow near a mold?

> Let students think of all possibilities here.

Q. In the above story, suppose the plate was carefully closed and kept inside the incubator (with optimum temperature) instead of being left outside. What would have happened then?

> An incubator maintains a set temperature. Also, bacteria usually grow at a higher temperature than fungi. So, if the plate was kept in the incubator, the mold would have probably not entered or grown on the plate.

Fleming first identified the mold to be *Penicilium notatum.* He then grew the mold in 'culture medium' so that he could extract the penicillin from it. It was this penicillin which killed the microbes around the mold. The extract which contained penicillin would quickly get degraded, and hence was not effective for treatment. Fleming tried different experiments to purify stable (active) penicillin for almost 10 years. But he was not successful in his attempts.

Q. What do you think Fleming would have done then?

> Encourage the students to discuss and write all possibilities before they turn to the next page

Story continues….

Howard Florey and Ernst Chain were scientists at Oxford university. In 1939, they borrowed Fleming's mold. They used their knowledge of pathology and biochemistry to extract the penicillin from it. They purified penicillin by performing various experiments. They injected the extract into mice infected with another highly infectious bacterium. In their experiment, 50 % of all the mice infected with the bacterium received penicillin and the others did not.

Q. Why were only 50 % of the mice given penicillin?

> The other 50 % of mice, which did not receive penicillin were the 'controls'. In any scientific experiment, a control group is important to prove that the intervention (here, penicillin,) causes the desired effect and that the effect may not be observed when there is no intervention. In the method of science, it is important to also consider a group with no intervention and so that we are sure that the outcome of the experimental group is a result of the intervention only.

Q. What do you think happened to:

a) Mice which received penicillin

b) Mice which did not receive penicillin

Q. Why was the experiment not tried directly in humans instead of mice?

> Before testing any drug on humans, it is usually tested on animals such as mice. These animals are specially bred for the purpose of laboratory research and are different than the ones which visit our homes! Mice that are bred for research are easy to handle, have small gestation periods (19-20 days, as against 9 months in humans) and produce a litter of 5-8 young ones at a time. Importantly, humans genes are very closely related to those of mice. All these factors make mice the most suitable option as model animalsIf the toxicity of a drug is not known, would somone try it directly on humans?
> No. But then why try it on mice either? Why kill mice?
> This is an ethical question and students might argue about the use of animals in experiments. There are authorities which regulate the use of experimental animals. They have a check on how many animals to be used, what is the experiment that will be done, etc.

Story continues….

Only in 1941, for the first time, penicillin was used as an antibiotic in the treatment of a patient. This patient had a bacterial infection as he got bruised with rose thorns on his face. He was given penicillin to treat the infection. But, he died as there was not enough penicillin to cure him completely. The need at that time was to produce penicillin in large amounts.

Image:PBS Newshour The real story behind penicillin

To obtain penicillin in large amounts, another scientist- Norman Heatley in Dr. Florey's lab practically used all available containers of all sorts, even bedpans and bottles to grow the mold *Penicillium notatum* in large volumes (litres). In spite of these procedures, the penicillin produced was not enough. Florey and Heatley were in search of ways to increase penicillin production.

Did you know, that the clue for excess production of penicillin came from a spoiled fruit??? Can you find out the name of this fruit. (Hint: It is a summer fruit)

M_ _ _ _ _ L _N

War conditions had made research difficult in England. So, just before World War II, Florey and Heatley went to America so that they could produce penicillin on a large scale. One day, a laboratory assistant brought the above fruit (M_________) to the lab which was covered with a 'golden mold'. This mold turned out to be a close relative of *Penicillium notatum,* (the green mold observed by Fleming) This particular mold was called *Penicillium chrysogenum* and it yielded 200 times more penicillin than *Penicillium notatum*, the earlier mold!

The wonder drug- Penicillin proved successful in saving the lives of many injured soldiers from bacterial infections during World War II.

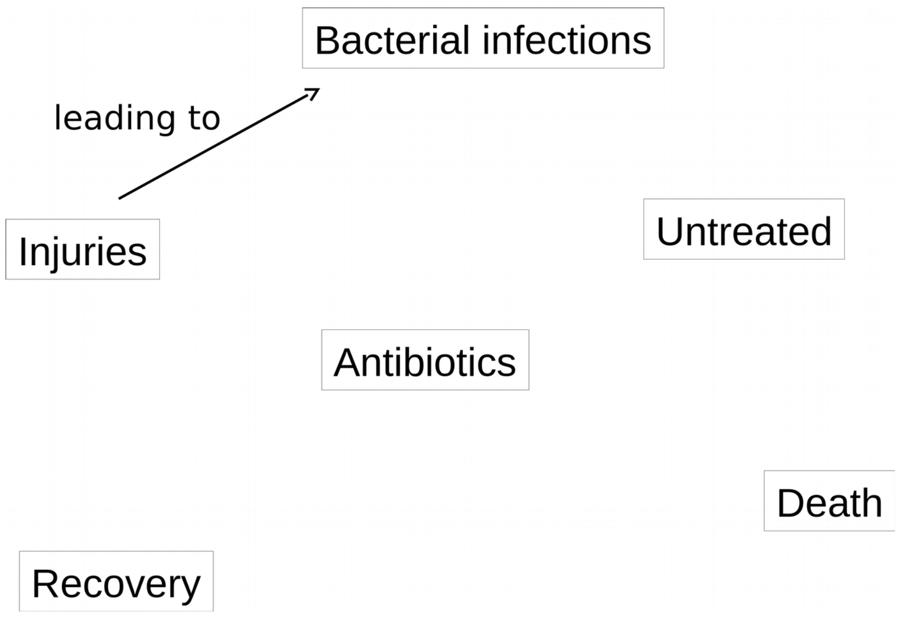
Q. When we see a fruit with a fungal growth, we usually throw it. Why did Heatley thought of using it for penicillin extraction?

> The burning problem at that time was producing penicillin in large amounts. Heatley was careful in observing and sensing the potential of that mold for increasing penicillin production.

Q. Imagine you are a part of the Nobel Prize Committee. Now that you have read the story, who all would you award the Nobel Prize for discovery of penicillin and why?

> This is an open-ended question
> The Nobel Prize was actually awarded to Alexander Fleming, Howard Florey (Pathologist) and Ernst Chain (Biochemist).
> The Nobel Prize is can be shared by not more than 3 people at a time.

Now, perform a task:
Make a map connecting the words below using arrows (→). On the arrows, write the appropriate linking phrases. You may choose the linking phrases from these: **'if left', 'may lead to', 'could result in', 'if treated with'** or any other phrase you find appropriate. The first one is done for you



Q. In what ways did the war affect the process of this discovery?

> Expected answers: funding for research, availability of space for carrying out experiments, availability of manpower, chemicals, resources, etc.

Q. How did penicillin help save millions of lives during the war? Does penicillin help in healing of wounds?

> During the world-war, people could have died more of bacterial infections caused due to the wounds, than the wound itself. So, penicillin helped in preventing infections in wounded soldiers.

How will you define antibiotics ?

> Antibiotics are produced by micro-organisms (fungi or bacteria) against the other competing micro-organisms.

Take-home message for you:

Complete the task using arrows as you did before to connect the words with arrows. You may use linkage words like 'can make', 'lead to', 'need to', 'can be', or any other phrase you may feel appropriate.

Work together

Mistakes

Scientists

Discoveries

Further challenges

Accidental

Once the students have solved all questions, teacher can discuss further questions (in discussion mode, writing not needed) with students.

Questions for discussion towards the end

We strongly recommend discussing these questions. If not in the same, then at least in another period. Most of these questions are open-ended. Teachers may invite all possible answers/arguments from students and encourage students to discuss them.

1. What different take home messages did the exercise give you?
   (They may be asked about effect of chance, accidental discoveries, working in teams)
2. Why did we do this exercise on ‘history of antibiotics’? Why is it significant now?
3. Bacteria existed even before 1928, so did fungi. So, what exactly happened in 1928, which triggered the process of purification of penicillin? (the chance event of bacteria and fungi growing on the same plate accidentally)
4. What if there was no upcoming war when penicillin was discovered?
5. If there were so many scientists involved, why is only Fleming’s name so popular and not others’?