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Answers

Microbiology: Discovering antibacterial agents

Worksheet 1: Antimicrobial resistance

1. Read information provided by your teacher on antibiotic resistance or go to the following webpages:
   https://www.fda.gov/consumers/consumer-updates/combating-antibiotic-resistance
   https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

2. What leads to antibiotic resistance?
   Every time antibiotics are used, there is a chance of bacteria evolving resistance, so they should only be used when necessary.

3. When should antibiotics be used? Fill out the table provided.

Situation	**Antibiotics?**	**Why?**
I feel sick, but I haven't seen a doctor	No	You don't know whether your infection is bacterial or caused by something else like a virus. Even if it's bacterial, you don't know without tests which antibiotic could be effective.
I have seen a doctor, and I have a viral or fungal infection	No	Antibiotics do nothing for viral or fungal infections.
I have seen a doctor, and I have a bacterial infection, but they don't recommend antibiotics because the infection is mild and will resolve on its own	No	Antibiotics don't work instantly. If your illness is mild and already being cleared by your immune system, adding antibiotics won't make you get better faster and might make you feel worse due to side effects.
My doctor recommends that I take antibiotics	Yes	Your doctor will recommend antibiotics when needed for a serious bacterial infection or high-risk situation (like abdominal surgery) and can tell you which antibiotic will be effective

4. Aside from resistance, what other consequences are there of taking antibiotics when not necessary (e.g. for a viral infection or mild bacterial infection)?
   Side effects. Antibiotics are medications with potential side effects like headache or diarrhea. If you take antibiotics when you don't need to, you could land up feeling MORE SICK than before due to the side effects! Antibiotics can also kill the healthy bacteria in your gut, leading to digestive problems.

5. In many countries, it is illegal for pharmacies to sell antibiotics without a prescription from a doctor for these reasons. Is this allowed in your country? Do you agree?
   _____
   _____
   _____
   _____

6. Do you know of any other unnecessary uses of antibiotics that could contribute to resistance?
   Agricultural use. Antibiotics are sometimes given to livestock to increase their grown rates. Ask students whether they think this should be allowed.

7. What about using antibiotics in science experiments, could that lead to antibiotic resistance? If so, what can we do to prevent this?
   Yes. Ensure that all antibiotics and antibiotic-exposed bacteria are properly disposed of so they do not escape into the environment.

8. What medical conditions or procedures could become dangerous if we run out of effective antibiotics?
   Not just infections like TB and tonsillitis but also conditions or procedures with a high infection risk, like cystic fibrosis, organ transplantation (which requires immune suppression), chemotherapy (which often leads to immune suppression), appendicitis, and many surgical procedures (which carry a risk of introducing infection), including tumor surgery and C-sections.

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Worksheet 3: Developing new medicines

1. Fill out table 1 for the substances you're intending to test. You can leave any answers you don't know blank. Note that it should be easy to look up the active substance in a product like mouthwash or cleaning products, but this can be very challenging for natural sources like plants, which may contain many potential active substances. Examples answers below. You can look at Refs [8,9] in the main article. Ref [8] reports in vitro results (i.e. in petri dishes not clinical tests in animals or people) only! Please read the conclusion.

Substance	Proposed active compound?	Expect antibacterial activity?	Why?	Is it toxic?
Fresh garlic juice	Allicin	Yes	Folk belief, in vitro results	Not at the concentrations normally eaten in food
Tea tree oil	Terpinen-4-ol	Yes	Folk belief, in vitro results	Yes
Cinnamon	Cinnamaldehyde+ various others (with plants, there is generally a mix)	Yes	In vitro results (See Ref [8])	Some compounds in cinnamon are toxic at high doses, e.g., coumarin.
Lemon juice	Citric acid	Not all bacteria?	Lemon juice can go bad	Not at the concentrations normally eaten in food
Alcohol mouthwash	Ethanol	Yes.	Product label/product testing	Yes, especially in higher doses
No ethanol mouthwash	Chlorhexidine gluconate 0,12%	Yes	Product label/product testing	Yes; can cause stomach irritation when ingested.
Honey	polyphenolic compounds, hydrogen peroxide, methylglyoxal, antimicrobial peptides	Yes	In vitro results (See Ref [9])	Not at the concentrations normally eaten in food

Table 1

Discussion questions

Answer the following questions for the substances you tested. If you don't know the answers for questions 6–8, then just say so. In science, recognizing what you don't know is as important as what you do know.

1. Are the results similar to what you hypothesized?
   _____
   _____
   _____
   _____
2. Which substance has the highest antimicrobial activity? And the lowest?
   _____
   _____
   _____
3. What effect did different dosages have? Are the inhibition zones similar or different?
   _____
   _____
   _____
4. For the foods, how do you think the concentrations you used compare to the concentration in your body after eating a normal portion of this food (even if it is all absorbed)?
   Unless the samples are diluted A LOT, the concentrations used are much higher than you would expect in the body after eating a portion of the food. You can look up the volume of water in a human body to make a rough estimate of the final dilution in the body assuming the substance survives digestion and is all absorbed (which it almost certainly won't be).

5. Could some of the nontoxic food substances be toxic if dosed in the body at the high concentrations you used?
   Probably. The dose makes the poison and most chemical compounds found in foods can be toxic if you eat enough of them. Essential oils are a good example. They are fine in the small quantities found in herbs and spices but many essential oils are toxic if ingested pure.

6. Do you know if the active substance is absorbed into the blood if ingested?
   For most substances, the answer will have to be "I don't know".

7. Is the active substance water soluble?
   For essential oils (which are also the active substances of many spices), no.

8. Is the active substance broken down by the digestive system or liver?
   For most substances, the answer will have to be "I don't know". Antimicrobial peptides, such as those found in honey, are probably broken down during digestion.

9. Based on the above answers, do you think the substances tested could be used as antibiotic medicines?
   For the known toxic substances, no. For the food derived substances, the most optimistic possible answer is "I don't know, the answers to the above questions and more testing would be needed". In most cases, probably not, since they have already been tested and the results were not encouraging or it is known that they are toxic at the effective dose or insoluble or broken down by digestion or not absorbed well. Just because they can't be used as antibiotics doesn't mean they aren't useful antimicrobials though. For example, honey can be used as an antibacterial wound dressing, and spices may help slow food spoilage.