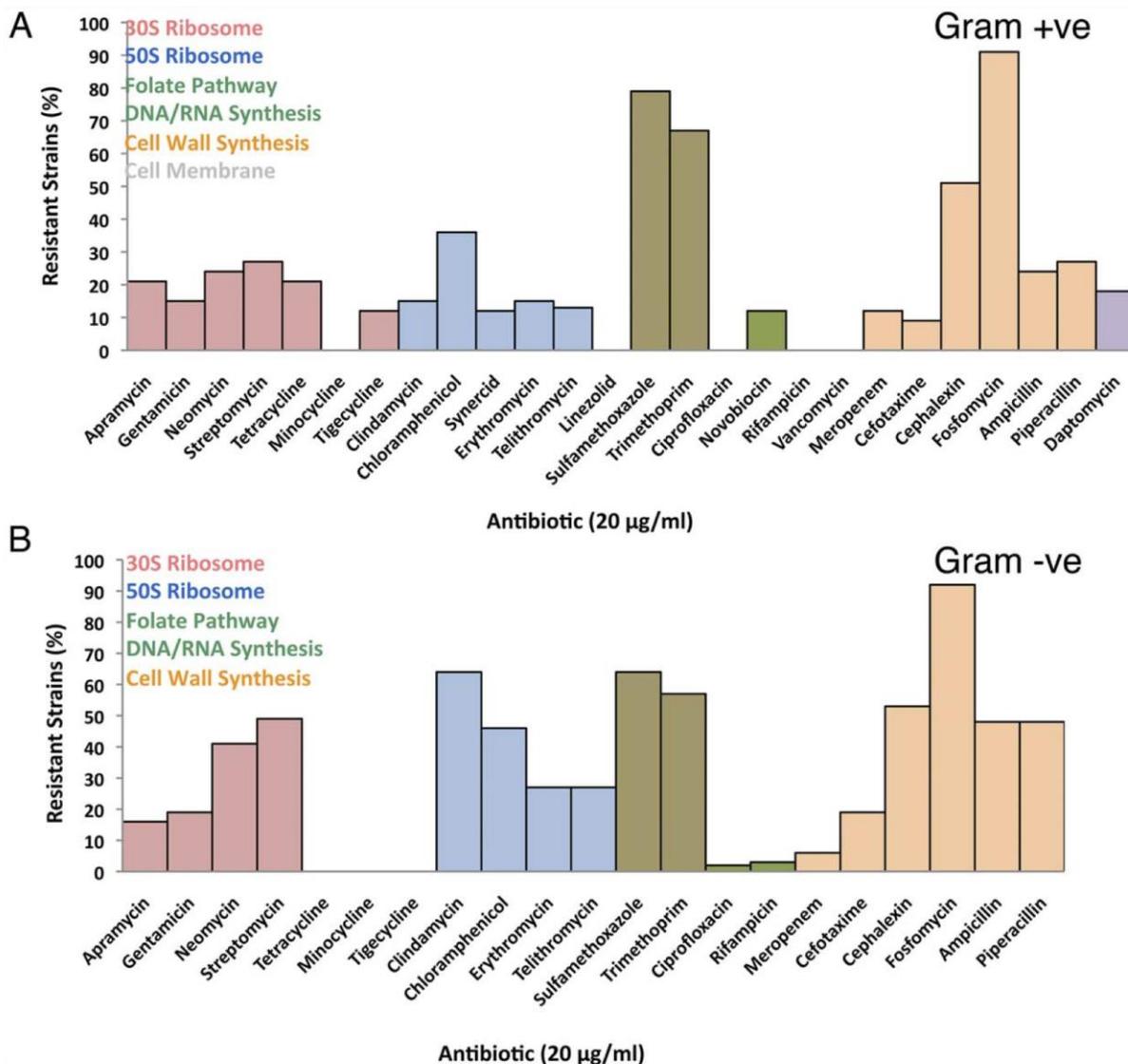




## HOW TO USE THIS RESOURCE

Show the figure below to your students along with the caption and background information either by printing the student handout or by projecting the image and reading the text aloud. The “Interpreting the Graph” and “Discussion Questions” sections provide additional information and suggested questions that you can use to guide a class discussion about the characteristics of the graph and what it shows.



Caption: Different strains of bacteria found in Lechuguilla Cave in New Mexico were tested for resistance to a variety of antibiotics. The antibiotics are grouped by color to show the similar mode of action/target they employ to kill bacteria. (A) Resistance in Gram-positive strains of bacteria; (B) Resistance in Gram-negative strains of bacteria.

## BACKGROUND INFORMATION

Since the discovery of the first antibiotic, penicillin, in 1928, antibiotics have revolutionized the fight against bacterial infections. Antibiotics are chemicals that specifically target and kill bacteria. Unfortunately, they are often prescribed unnecessarily and frequently misused by patients. Research has shown that these trends have contributed to the recent evolution of bacteria that are resistant to antibiotics. What is less clear is whether the bacterial genes that confer antibiotic resistance (collectively called the “resistome”) are all newly evolved since the introduction of antibiotics or have evolved independently.

To answer this question, scientists collected bacteria from locations that had never been exposed to humans or modern antibiotics. Their study site, a remote cave located within Carlsbad Caverns National Park in New Mexico, called Lechuguilla Cave, was estimated to have been isolated from human contact and surface contamination (such as rainwater) for at least 4 million years, with extremely restricted access since its discovery in the 1980s. The scientists collected over 500 unique bacterial strains from three locations in the cave. Of these, 93 culturable strains were randomly selected to be screened for resistance to modern-day antibiotics.

About 60% of the bacteria were Gram-negative, which means that they have a protective outer membrane and are resistant to many antibiotics. The remaining bacteria were classified as Gram-positive, meaning they lack this outer membrane. The bacteria were tested for survival against 26 different antibiotics, each falling into one of six categories based on the protein or pathway it targeted within bacterial cells: 30S ribosome, 50S ribosome, folate pathway, DNA/RNA synthesis, cell wall synthesis, or the cell membrane. Each strain of bacteria was tested by placing it on growth medium (food) that contained a high concentration of an antibiotic. If the bacterial strain could reach a cell density (a measure of bacterial growth) of at least half that achieved by the same strain growing without antibiotic, it was considered resistant to the antibiotic.

## INTERPRETING THE GRAPH

This figure has two bar graphs that each show the percentage (on the y-axis) of Gram-positive (A) or Gram-negative (B) strains that are resistant to a particular clinically used antibiotic. (The antibiotic names are labeled on the x-axis.) About 63% of the bacteria tested were Gram-negative, which means that they have a cell wall that is more resistant to certain classes of antibiotics than the cell walls of Gram-positive bacteria. For this reason, the Gram-negative bacteria were only screened for resistance to antibiotics with demonstrated activity against Gram-negative strains (21 in total). In contrast, the Gram-positive strains were screened against 26 antibiotics. The targets of the 26 different antimicrobial agents are categorized and shown in different colors.

Only one antibiotic, minocycline, effectively killed 100% of all the bacterial strains tested. Conversely, roughly 90% of the strains tested were resistant to fosfomycin. This suggests that our arsenal of antibiotics has very few lines of defense left against the antibiotic resistance genes that exist in the environment.

The figure shows that these bacteria, which have never been exposed to modern-day clinical or agricultural sources of antibiotics within the last century, nevertheless demonstrate resistance to many modern antibiotics. And not only are these bacteria resistant to diverse antibiotics, but the resistant bacterial strains represent high diversity as well. These observations suggest that antibiotic resistance genes likely originated millions of years ago and have diversified greatly in the time since. They also suggest that human overuse and misuse of antibiotics are not solely responsible for the emergence of antibiotic resistance.

To understand why antibiotic resistance evolved so long ago, consider the life of a bacterium. To have the best chance of survival, a bacterium must outcompete its neighbors. One way of doing this is to produce antibiotics. The bacteria that produce antibiotics must naturally be resistant, but the susceptible strains may also develop resistance over time.

**Teacher Tip:** Prompt your students to explain the parts of the graph as applicable:

- Graph Type: Bar Graph
- X-axis: Types of antibiotics
- Y-axis: % of bacterial strains with resistance to a particular antibiotic

#### DISCUSSION QUESTIONS

- What similarities and differences do you notice between the two graphs?
- Which antibiotics were the most successful at killing the cave bacteria? Which were the least successful? What evidence supports your claims?
- Does this data support a hypothesis that some resistance genes evolved in bacteria that weren't exposed to modern antibiotics? Why or why not?
- Did any antibiotics affect Gram-positive and Gram-negative strains very differently? What is a possible explanation for this?
- Which feature (antibiotic target) of bacteria seems to be the most resistant to antibiotics? Why might that be?
- Thinking about the cellular features that antibiotics target, why do you think that antibiotics only harm bacterial cells and not your own human cells?
- From what other environments might scientists collect bacterial samples to further test their hypothesis that antibiotic resistance is a trait that evolved without being influenced by modern antibiotic use?
- What precautions might need to be taken by the scientists who work with these bacteria in the lab?
- How many of the 26 antibiotics successfully killed 100% of the bacterial strains (both Gram-positive and Gram-negative)? What does this make you think about our ability to fight infections with our current arsenal of antibiotics?
- Do the results of this study give us any insights into how we might combat antibiotic resistance going forward? If so, what?

#### SOURCE

Figure 2 from:

Bhullar K., Waglechner N., Pawlowski A., Koteva K., Banks E.D., *et al.* (2012) Antibiotic Resistance Is Prevalent in an Isolated Cave Microbiome. *PLoS ONE* 7(4): e34953. doi:10.1371/journal.pone.0034953

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