Cell Wall Structures and Antibiotic Action

Resource Type: Curriculum: Classroom

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Abstract

This problem-based exercise challenges students to think critically about the connection of antibiotics to cell wall structure. Without prior knowledge of antibiotics' modes of action, students are asked to deduce how the differential components of bacterial cell walls create an observed antimicrobial activity on a panel of microorganisms. Our format employs three distinct approaches to frame student understanding: first, small groups of 10 to 15 students provide a space for active discussion and peer teaching; second, students apply their knowledge to solve an activity based on an experimental scenario; finally, the process of lecture, discussion, and small groups is iterative.

Activity

Description: A small group activity which uses a problem-based approach to introduce students to the differential action of antibiotics on the components of bacterial and archaeal cell walls.

Audience: Allied health majors, Microbiology/biology majors

Microbiology Keywords: Antibiotic resistance, Antibiotics, Case Studies, Cell wall

Pedagogy Keywords: Problem-based learning

Science Discipline Keywords: Bacteriology, Microbiology

Core Themes: Theme 1: Microbial cell biology, Theme 6. Teaching and learning

Core Skills: Thinking: Analysis

Learning Time: 1 hour in class with homework assigned prior to the class

ACTIVITY

Learning Objectives

At the completion of this activity students will be able to:

1. describe the function of various bacterial and archaeal cell wall structures.
2. relate cell wall structure to the effectiveness of antimicrobial compounds.
3. explain that cell membranes are common to all bacteria and archaea and can be a target for antimicrobial compounds.

Background

Students are given a review sheet before the class in which the small group activity occurs. This sheet covers the basic cell wall structures for gram-positive and gram-negative bacteria and archaea. From this review or from their class materials, students should be able to identify, describe, and label the appropriate structures on a model cell wall using the following terms:

cell wall
cytoplasmic membrane
membrane-bound proteins
outer membrane
lipopolysaccharide
lipoteichoic acid
teichoic acid
porins
periplasmic space

PROCEDURE

Materials.

One worksheet for every student

Student Version.

Student Version
Sample of Student Work

Instructor Version.

Instructor Version

Safety Issues.

None

Suggestions for Determining Student Learning.

The primary mechanism for determining student learning is the answers they provide to the questions on the worksheet. We have also used as a secondary assessment tool a pre- and posttest given to students the first and last day of lab recitation (see field testing section for details).

Field Testing.

During the spring of 2007, we developed a pre- and posttest that was given to students at the first lab recitation session before any small group or lab activity covered the material being tested. This same test was also given to students on the last day of lab recitation. With this design, our data provides insight into student learning and the level of material retention. Using questions 1, 4, and 5 from the test, we quantified student learning on the function of the cell wall and antibiotics. Test scores for question 1 improved from 68% (n = 75) to 90% (n = 71), scores on question 4 improved from 68% (n = 75) to 90% (n = 71) where scores for question 5 improved from 61% (n = 75) to 92% (n = 71).

Student Data.

None available at time of submission.

SUPPLEMENTARY MATERIALS

Possible Modifications.

This exercise could be modified in at least two different ways. If using it with majors in an upper-level course, the “helpful background information” can be removed and the description of each unknown antibiotic could be changed to the specific site of action of each antibiotic. In this way, the activity could cover the modes of action of the antibiotics at a greater molecular level. If working with allied health students, the focus of the activity could be changed to reflect the differences between eukaryotic and prokaryotic cells and how antibiotics specifically target one and not the other.

References.

1. **Foster, J. W., and J. L. Slonczewski.** 2008. Cell structure and function, p. 82–97. *In* Microbiology an evolving science, 1st ed. W. W. Norton and Co., New York, NY.

2. **Madigan, M. T., and J. M. Martinko.** 2005. Cell structure and function, p. 66–81. *In* Brock biology of microorganisms, 11th ed. Pearson Prentice Hall, Upper Saddle River, NJ.

3. **Paustian, T.** 2001. More cell wall. http://lecturer.ukdw.ac.id/dhira/BacterialStructure/MoreCellWall.html.

4. **Paustian, T.** 2002. Cell wall. http://lecturer.ukdw.ac.id/dhira/BacterialStructure/CellWall.html.

5. **Schaechter, M., J. L. Ingraham, and F. C. Neidhardt.** 2006. Prokaryotic cell structure and function: envelopes and appendages, p. 22–31. *In* Microbe. ASM Press, Washington, DC.

Answer Keys.

Provided in the Instructor Version

Appendices.

Review Sheet
Student Version
Instructor Version
Pre- and posttest

Small Group Activity
Cell Wall Structures and Antibiotic Action Review

To prepare, review sections 4.5, 4.6, 4.8, and 4.9 in Brock Biology of Microorganisms, 11th ed.

Part I

1) Look at the diagrams on the next page of three different prokaryotic cell envelopes. Determine which figure shows a gram-positive, a proteobacterial or gram-negative, and an archaeal cell envelope.

2) Label the components of each cell envelope using the list below. (Note: not all cells have all the structures listed.)

cell wall	lipoteichoic acid
cytoplasmic membrane	periplasmic space
membrane-bound proteins	porins
outer membrane	teichoic acid
lipopolysaccharide	

3) Answer the following questions:

a) Which structure is the true permeability barrier? What would happen to the cell if you made the membrane "leaky"?

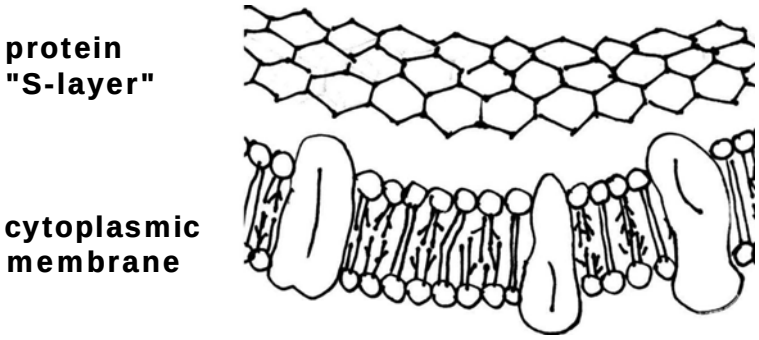
b) Which structure provides support and shape? What would happen to the cell if you broke this structure?

c) Which structure only allows hydrophilic small molecules (<600 daltons molecular weight) through? Where are these found? Which organisms have them?

d) What happens if you treat a bacterial cell with lysozyme under isotonic conditions? What is this type of cell called?

Cell wall type _____

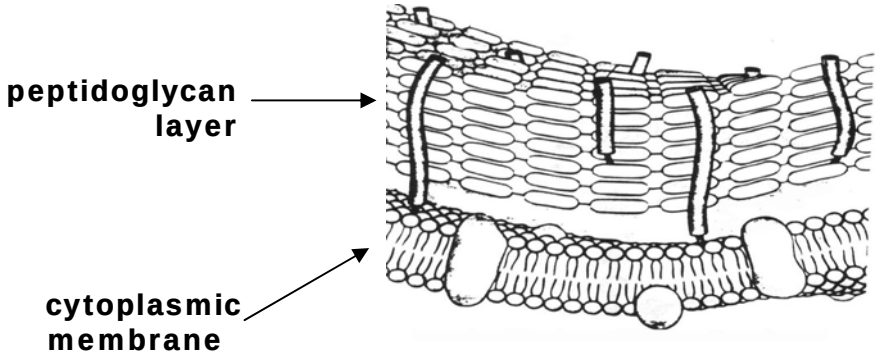
outside cell



inside cell

Cell wall type _____

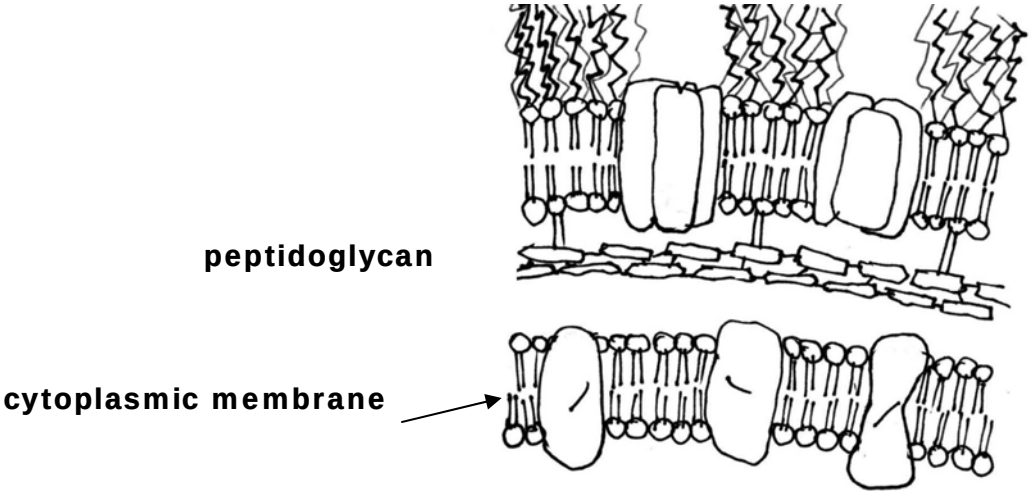
outside cell



inside cell

Cell wall type _____

outside cell



inside cell

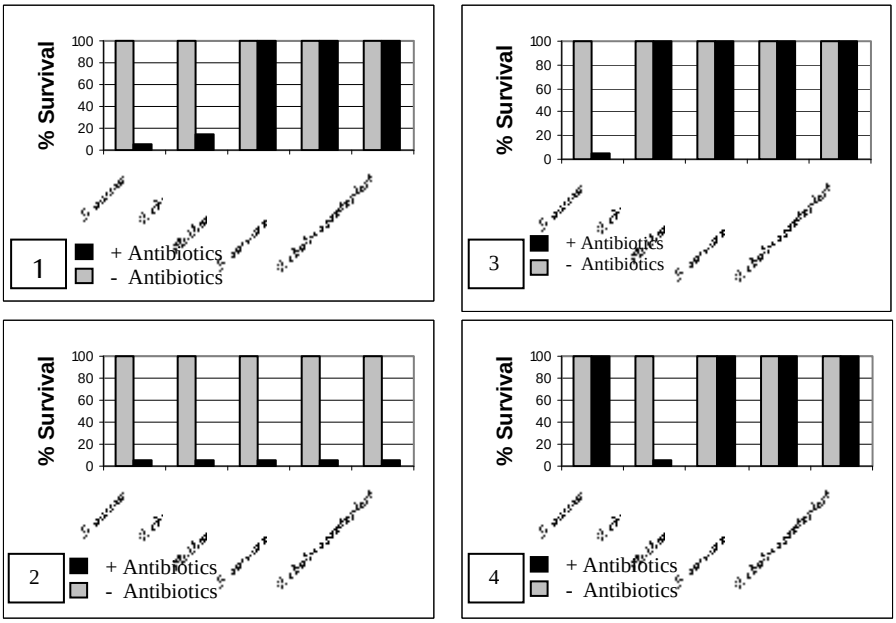
Small Group Activity—Cell Wall Structures and Antibiotic Action

You have recently been hired as an Assistant Professor where your research deals with isolating novel antibiotics to aid in the growing problem of antibiotic resistance. In your first months of work, you have isolated several interesting antimicrobial compounds which show real promise. Unfortunately, late one night you did not label the tubes clearly. Now your promising work needs to be pieced back together with only a few scraps of information scribbled on a napkin from that night. Good luck!

Deciphered napkin scribblings…

Antibiotic A: 0.5 kDa protein, targets peptidoglycan *983-7554 cutie from Castaways*
Antibiotic B: 20 kDa protein, targets peptidoglycan
Antibiotic C: Cationic antimicrobial peptide *get!! milk, bread, cheese, stamps*
Antibiotic D: Targets lipopolysaccharide *Bus #81 comes at 7:10*

With some more searching, you found these results in your notebook. Unfortunately they are not labeled either. These graphs represent percentage of survival of bacteria or protoplasts after treatment with the antibiotics.
Samples with antibiotic added (black bars); controls with no antibiotic added (grey bars)



Helpful background information

Staphylococcus aureus: gram-positive bacterium *Vibrio cholera*: gram-negative bacterium
Methanosarcina: an archaean bacterium

Cationic antimicrobial peptides (CAMPs): these positively charged antibiotics are attracted to the negatively charged cell wall and membrane. They are hydrophobic, and they insert into the membranes to create pores.

Small Group Activity—Cell Wall Structures and Antibiotic Action
Writing Assignment

Your name:
Your instructor's name:

Complete the following questions, limiting your answers to the spaces provided.

1. **Using the information on the previous page, match each antibiotic with an experiment. Fill in the blanks below and briefly discuss the results observed for each experiment, making sure to include the predicted mode of action for each antibiotic. (9 points)**

Experiment #1 is Antibiotic _____

Experiment #2 is Antibiotic _____

Experiment #3 is Antibiotic _____

Experiment #4 is Antibiotic _____

2. **Pick one of the antibiotics above (A–D) and describe how a bacterial cell could become resistant to that antibiotic, i.e., what in the cell would have to change to make a bacterium resistant? (1 point)**

Small Group Activity
Cell Wall Structures and Antibiotic Action Review

To prepare, review sections 4.5, 4.6, 4.8, and 4.9 in Brock Biology of Microorganisms, 11th ed.

Part I

1) Look at the diagrams on the next page of 3 different prokaryotic cell envelopes. Determine which figure shows a Gram (+), a Proteobacterial or Gram (-), and an Archaeal cell envelope.

2) Label the components of each cell envelope using the list below. (Note: not all cells have all the structures listed)

cell wall
cytoplasmic membrane
membrane-bound proteins
outer membrane
lipopolysaccharide
lipoteichoic acid
periplasmic space
porins
teichoic acid

3) Answer the following questions:

a) Which structure is the true permeability barrier? What would happen to the cell if you made the membrane "leaky"?

b) Which structure provides support and shape? What would happen to the cell if you broke this structure?

c) Which structure only allows hydrophilic small molecules (< 600 daltons mol wt) through? Where are these found? Which organisms have them?

d) What happens if you treat a bacterial cell with lysozyme under isotonic conditions? What is this type of cell called?

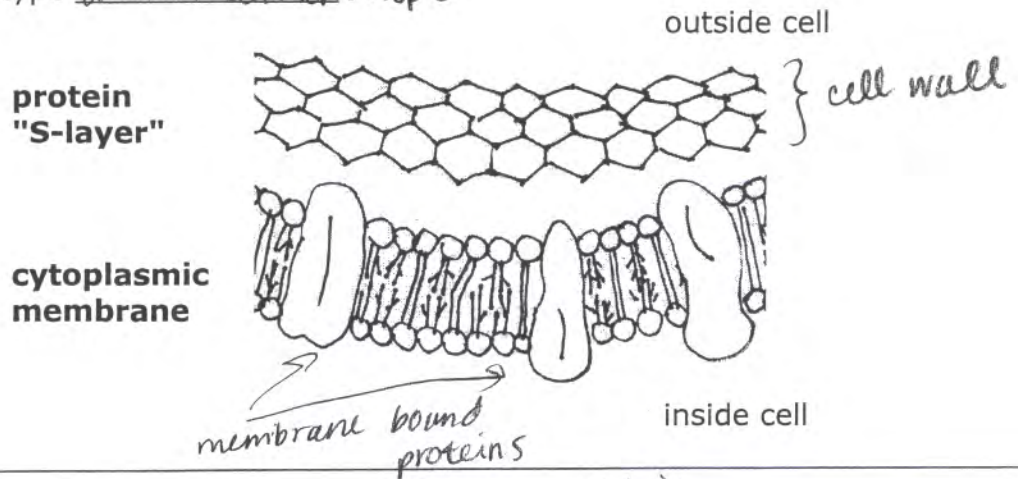
(a) the cytoplasmic membrane is the true permeability barrier because it control which substances can go in & out of the cell. If the membrane became leaky, intracellular components would leak out and prevend the cell from getting appropriate concentration gradients for important biochemical reactions

(b) the peptidoglycan provide shape and support. If broken, the cell would lose its rigidity and ~~possibly~~ ability to withstand high turgor pressure.

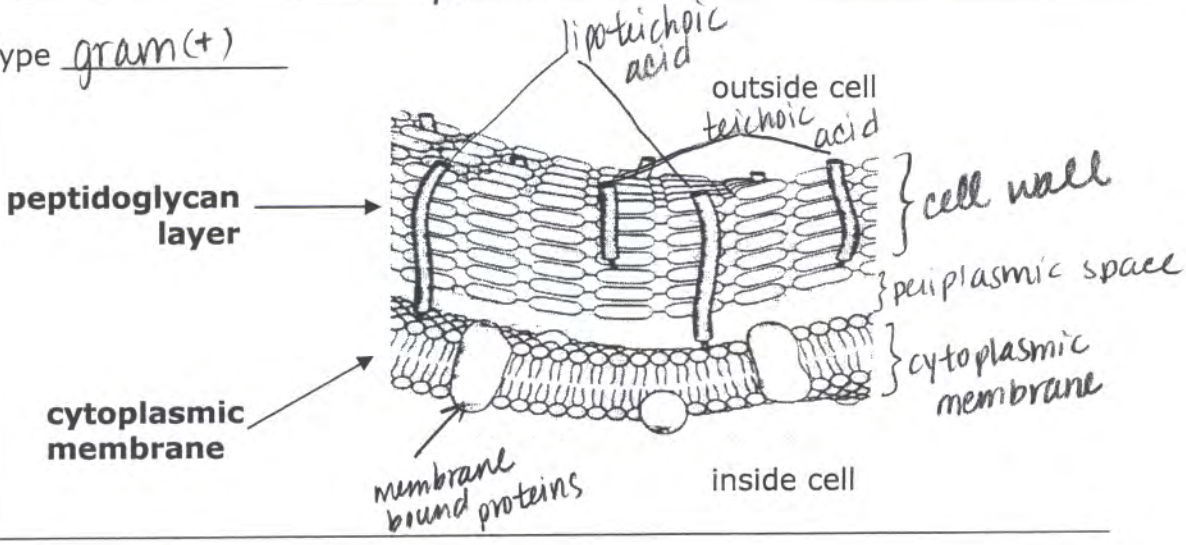
(c) porins - they are found in the outer membrane of gram (-) bacteria.

(d) lysozymes break down peptidoglycan. Under isotonic conditions, water does not enter the cell so the membrane remains intact. This cell becomes a protoplast

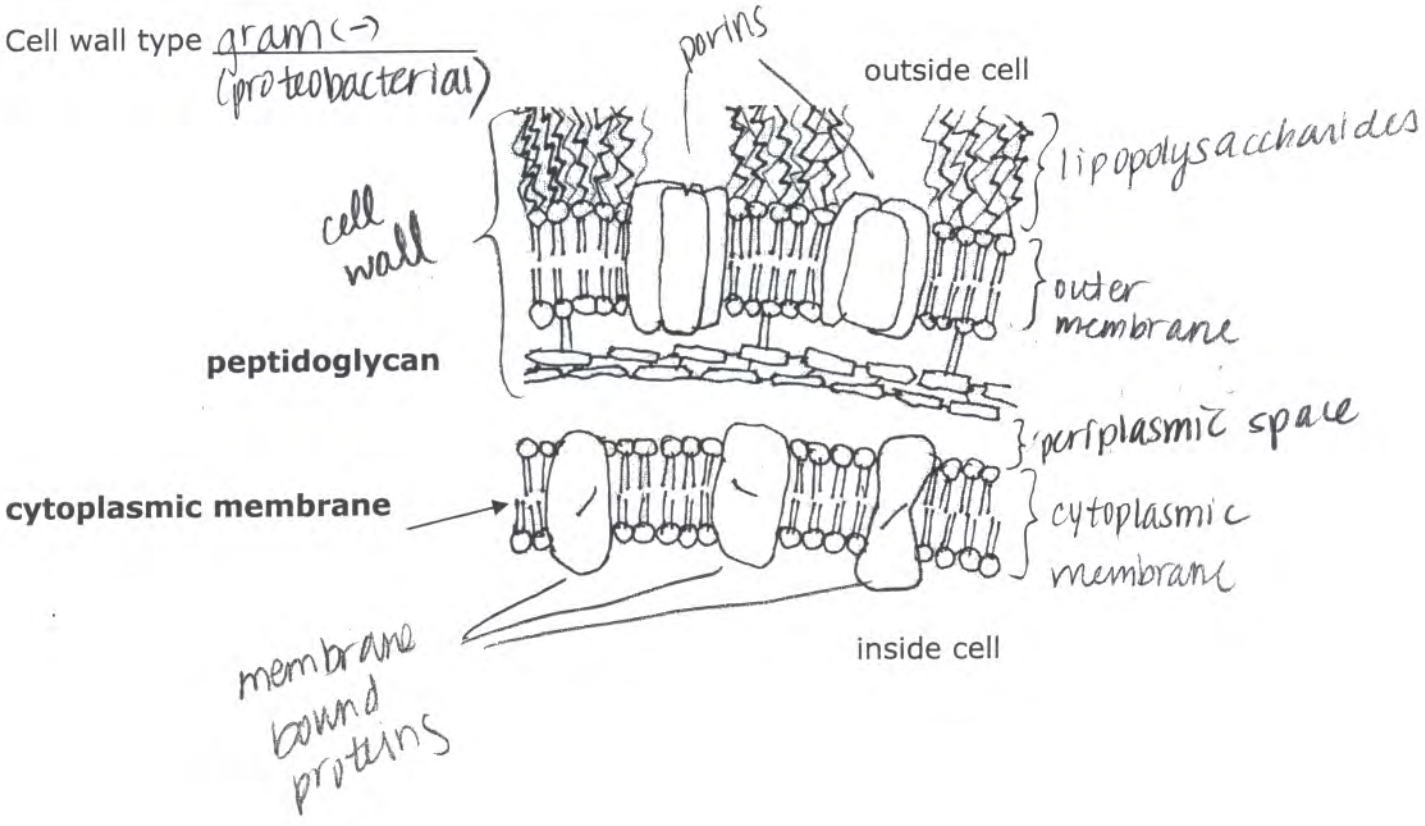
Cell wall type archael cell envelope



Cell wall type gram (+)



Cell wall type gram (−) (proteobacterial)



Small Group Activity — Cell Structure and Function
Writing Assignment

Your Name: [redacted]
Your Instructor's name: [redacted]

Complete the following questions LIMITING your answers to the spaces provided

1. Usi ng the information on the previous page, match each antibiotic with an experiment. Fill in the blanks below and briefly discuss the results observed for each experiment, making sure to include the predicted mode of action for each antibiotic. (9 points)

Experiment #1 is Antibiotic A

It appears that only *S. aureus* and *V. cholera* are affected by the antibiotic. Both of these bacteria have peptidoglycan while *Methanobacterium* and the protoplasts do not. It appears that antibiotic A would cause these results since it attacks peptidoglycan. Its small protein size would allow the protein to move through the outer membrane to attack the peptidoglycan of gram(-) cells, while effectively attacking the peptidoglycan in gram(+).

Experiment #2 is Anitbiotic C

This experiment showed that archael, gram(+), & gram(-) cells were affected. Because antibiotic C consists of CAMP's, it will cause any of these cell types to be lysed. CAMPs cause pores to form in the cell membrane, which may cause water to move into the cell and rupture the cell, killing it.

Experiment #3 is Antibiotic B

In this experiment, only *S. aureus*, a gram(+) cell is affected. Gram(+) cells have a thick peptidoglycan layer that is exposed to the environment. Antibiotic B would have easy access to attack this layer possibly by breaking down the cross links that support the peptidoglycan structure.

Experiment #4 is Antibiotic D

In experiment 4, only the *V. cholera* is affected as its survival plummets after being administered an antibiotic. The only difference *V. cholera* has from the other organisms is that it is gram(-) and contains lipopolysaccharides. Antibiotic D attacks LPS and is the only antibiotic of the 4 to do so. By attacking the LPS, the antibiotic may cause the cell to burst as it may not be able to withstand the turgor pressure.

2. Pick one of the an tibiotics above (A-D) and describe how a bacterial cell <u>could</u> become <u>resistant</u> to that antibiotic. i.e. What in the cell would have to change to make a bacterium resistant? (1 point)

To become resistant to antibiotic D, gram(-) cells need to gain a mutation that alters the LPS slightly so that the links in LPS cannot be broken by the antibiotics.

Small Group Activity 1—Cell Wall Structures and Antibiotic Action

Before class, students should have completed the provided review sheet (part I) which covers the basic components of cell envelope structure of the microorganism presented in this activity. Typically, small group instructors review the answers to this sheet at the beginning of class to refresh students' memory and also to ensure the accuracy of their answers. After the review, distribute this activity and divide the class into groups of three students. Groups are encouraged to work together to determine the correct answers to the questions, however each individual needs to write his or her own reasoning as to why the answer is correct.

You have recently been hired as an Assistant Professor where your research deals with isolating novel antibiotics to aid in the growing problem of antibiotic resistance. In your first months of work, you have isolated several interesting antimicrobial compounds which show real promise. Unfortunately, late one night you did not label the tubes clearly. Now your promising work needs to be pieced back together with only a few scraps of information scribbled on a napkin from that night. Good luck!

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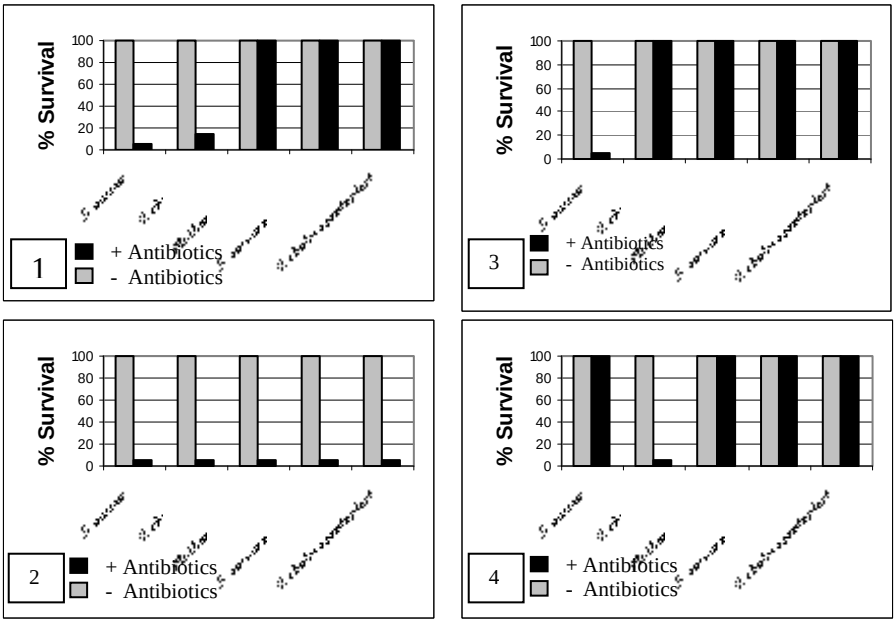

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Small Group Activity—Cell Wall Structures and Antibiotic Action
Writing Assignment

Your name:
Your instructor's name:

Complete the following questions, limiting your answers to the spaces provided

1. **Using the information on the previous page, match each antibiotic with an experiment. Fill in the blanks below and briefly discuss the results observed for each experiment, making sure to include the predicted mode of action for each antibiotic. (9 points)**

Experiment #1 is Antibiotic _____A___

Cell wall active antibiotic. The antibiotic in experiment #1 killed gram-positive and gram-negative bacteria and had no effect on protoplasts or archea. This can be explained by a low molecular weight antibiotic that can pass through the porins and attack the peptidoglycan of the gram-negative bacteria and degrade the thick peptidoglycan cell wall of the gram-positive bacteria causing lysis and death of bacteria. There is no effect on archea since it has pseudopeptidoglycan and protoplasts which have no peptidoglycan and therefore are not susceptible to the action of this antibiotic.

Experiment #2 is Antibiotic ____C___

This antibiotic killed all three microorganisms as well as the protoplasts, therefore it attacks the cell membrane. This is present in the cell envelopes of all three bacteria tested and is still present in protoplasts.

Experiment #3 is Antibiotic ____B____

This antibiotic killed only gram-positive cells. Therefore it is cell wall active, since it kills the gram-positive bacteria, but not the gram-positive protoplasts. It is a large antibiotic that cannot penetrate the outer membrane of the gram-negative cell (porins exclude large molecules) and therefore cannot access the peptidoglycan.

Experiment #4 is Antibiotic ____D___

This antibiotic killed only gram-negative cells. Lipopolysaccharide is only found in the outer membrane of gram-negative bacteria; therefore antibiotic D only attacks the gram-negative bacteria. The protoplasts are not affected since the cell wall has been removed.

2. **Pick one of the antibiotics above (A–D) and describe how a bacterial cell could become resistant to that antibiotic, i.e., what in the cell would have to change to make a bacterium resistant? (1 point)**

Any of the following that seems reasonable:

1. modification of the target (different side chains or bonds in peptidoglycan, change charge of cell wall or membrane to repel cationic peptide, etc.; any ideas that are reasonable are accepted)
2. efflux of antibiotic
3. inactivation of the antibiotic: degradation or modification of antibiotic
4. altered membrane permeability

Pre-post test **Your name**_____

Please answer these questions to the best of your ability. The results will not in any way be used to determine your grade, but provide us with background information so that we may present material at the most appropriate level for the class.

____ 1) In a nonisotonic solution, if the **cell wall** of a bacterium is removed, the cell will
A. be unaffected.
B. burst due to an influx of water into the cell.
C. lose motility, but otherwise be unaffected.
D. stop growing due to an efflux of solutes from the cell.

_____ 2) Which cellular process below is most often associated with the release of **acidic waste products**?
A. aerobic respiration
B. amino acid biosynthesis
C. fermentation
D. oxygenic photosynthesis

_____3) The electrons in an **electron transport chain** move from
A. negatively to positively charged molecules.
B. smaller to larger sized electron carriers.
C. molecules with lower to higher reduction potentials.
D. molecules that can pump protons to those that cannot.

4) In a respiring bacterium, if **proton pumping** across the cell membrane is stopped, ATP synthesis by the ATP synthase will:
_____Stop _____Increase _____Remain the same
Pick one answer and explain why.

____ 5) **Antibiotics**
A. affect either gram-negative or gram-positive bacteria, but not both.
B. always bind to the outside of a cell.
C. must be transported inside the cell to inhibit cell growth.
D. work by inhibiting a specific molecular target.

6) Compared to planktonic or free-floating cells, **bacterial cells in biofilms** are typically
_____ less susceptible to antimicrobial agents.
_____ equally susceptible to antimicrobial agents.
_____ more susceptible to antimicrobial agents.
Pick one answer and explain why:

_____7) In the **negative regulation of protein synthesis** in bacteria,
A. there is no inducer.
B. the regulatory protein binds the operator region of an operon.
C. the regulatory protein and RNA polymerase must both bind together.
D. a repressor prevents the ribosome from attaching to rRNA.

8) Match each mode of **horizontal gene transfer** with the statement that best describes it:

_____Conjugation
_____Transformation

A. A genetic element that can move from one place in the DNA to another
B. Movement and incorporation of free DNA into a bacterial cell
C. The process by which two separate DNA molecules become incorporated into one
D. Transfer of genes from one bacterium to another by a virus
E. Transfer of genes requiring cell-to-cell contact

_____ 9) Which method would be best to identify a **new emerging viral pathogen**?
A. Gram stain
B. Phase-contrast microscopy
C. The fluorescent in situ hybridization assay
D. Transmission electron microscopy

_____ 10) **Fluorescently-labeled antibodies** can be used to identify a specific type of bacterial cell in a mixed population because
A. antibodies kill off specific groups of bacteria.
B. only that type of bacterial cell produces a fluorescently labeled antibody.
C. that antibody binds only to a specific antigen on a bacterial cell envelope.
D. that antibody gets taken into the bacterial cell through special transporters.

11) Match each bacterium with its typical characteristic.

_____ *Bacillus*
_____cyanobacteria
_____*Pseudomonas*
_____*Staphylococcus*

A. Salt resistant bacteria commonly found on skin

B. Gram-negative rods that respire and are often resistant to antibiotics

C. Gram-positive rods that develop extremely resistant spore structures

D. Photosynthetic bacteria that are very similar to chloroplasts