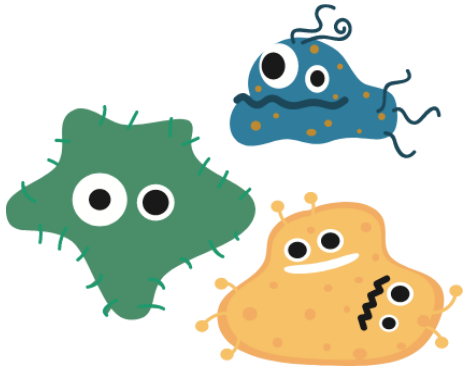
FINDING LIFE-SAVING MICROBES



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RI: Life Science Lab - BSC 1005L – 039

04/18/2018

The Antibiotic Crisis

- After penicillin's discovery, there was a breakthrough in antibiotic research.

- By the 1980s, many thought infectious disease was a thing of the past.

- After the 1980s, no new or different classes of antibiotics have been discovered (Pew Charitable Trusts, 2016).

- There has been an increasing level of bacterial resistance to antibiotics which is a severe threat to our global health.

http://www.smallworldinitiative.org/meme-contest-entries/

"If we don't act, antibiotic resistance will kill more people than cancer & diabetes combined by 2050"
(CDC, 2016)

Small World Initiative

crowdsourcing antibiotic discovery

- Students from all over the world are encouraged to use their local soil to try to discover new antibiotics.
- Billions of microbes - both good and bad - inhabit Earth.
- Most new antibiotics come from soil bacteria or fungi.
- In SWI, students dig up soil, isolate their bacteria, and test it for antibiotic characteristics.

Source:https://static1.squarespace.com/static/555cec2ae4b0902ed19d910b/t/5781e6f3c534a5d68f225213/1468131118335/Western+Alabama+Symposium+Cover.jpg?format=1500w

ESKAPE Pathogens, usually associated with hospitals, currently make up most of the antibiotic-resistant infections.

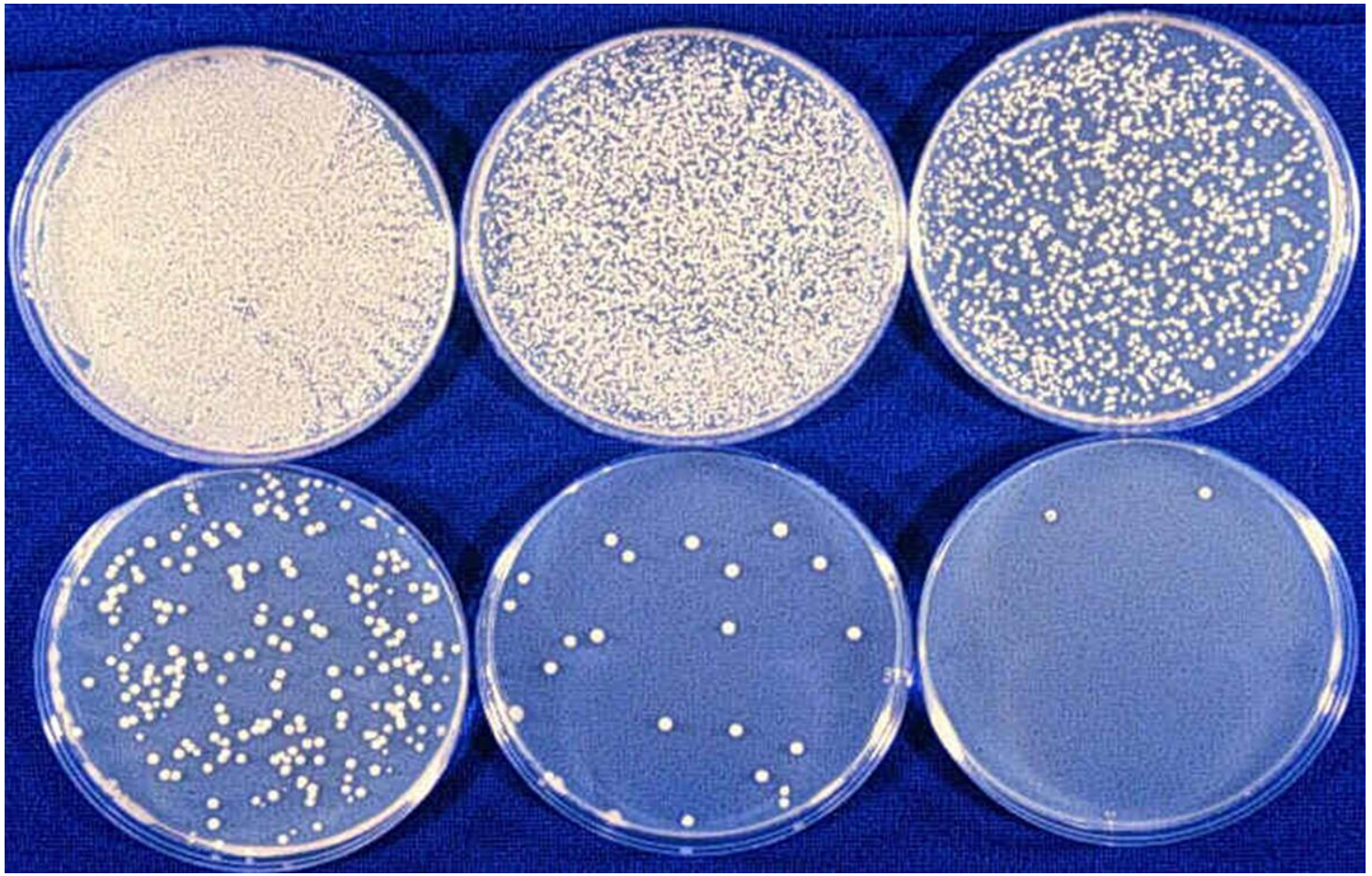
Enterococcus faecium
Staphylococcus aureus
Klebsiella pneumoniae
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacter species

Source: https://www.popsci.com/sites/popsci.com/files/styles/1000_1x_/public/images/2017/09/deposit_photo-_worldmap_pills_small.jpg?itok=BmWQy8EB&fc=50,50

SWI Experiments

- **Lab 1 -** There are billions of bacteria found in even a handful of soil. First, we each dug up some soil.

Lab 2 - In order to see our bacteria more easily, we had to **dilute** our soil. Our goal was to have at least one plate with countable colonies.

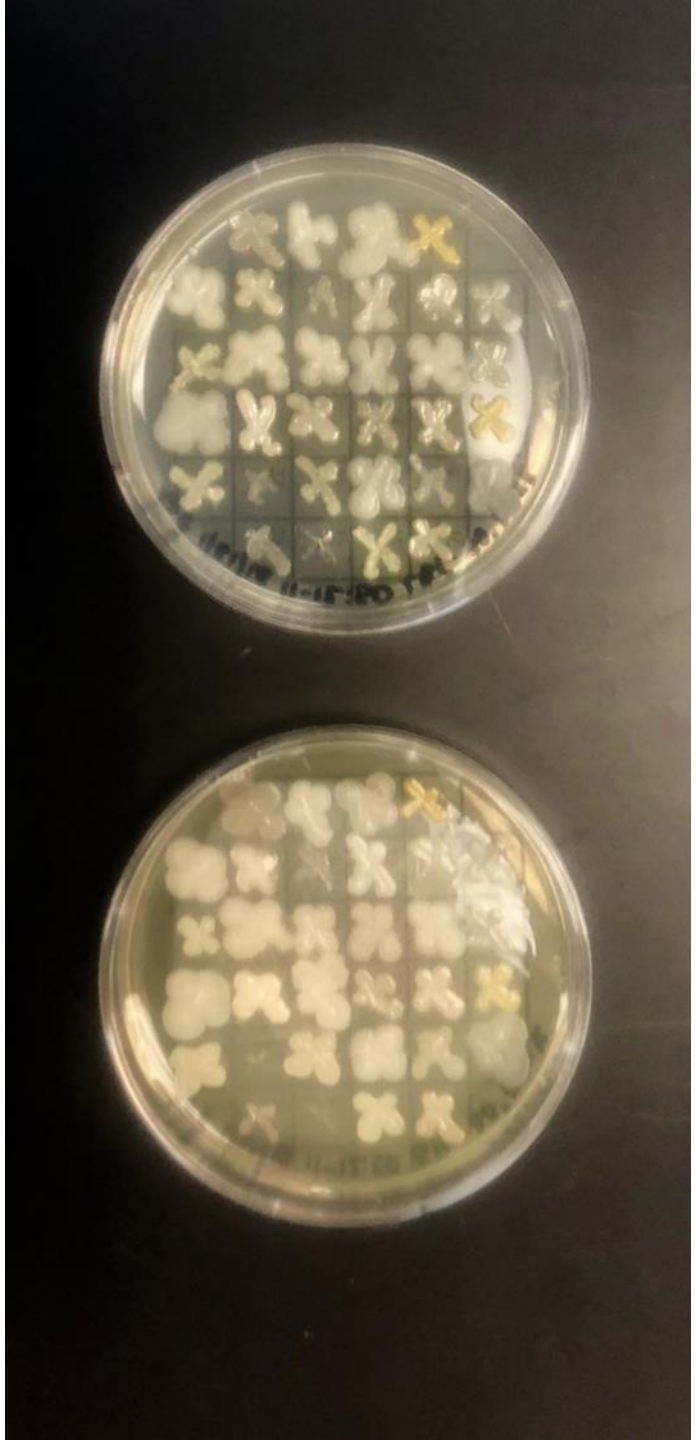


(Source: https://www.phys.ksu.edu/gene/photos/lab12a.jpg)

Pick and Patch

- **Lab 3 -** Picked and patched our bacteria onto two food plates - one LB and the other one of our choice.

- A single colony can contain thousands of bacteria

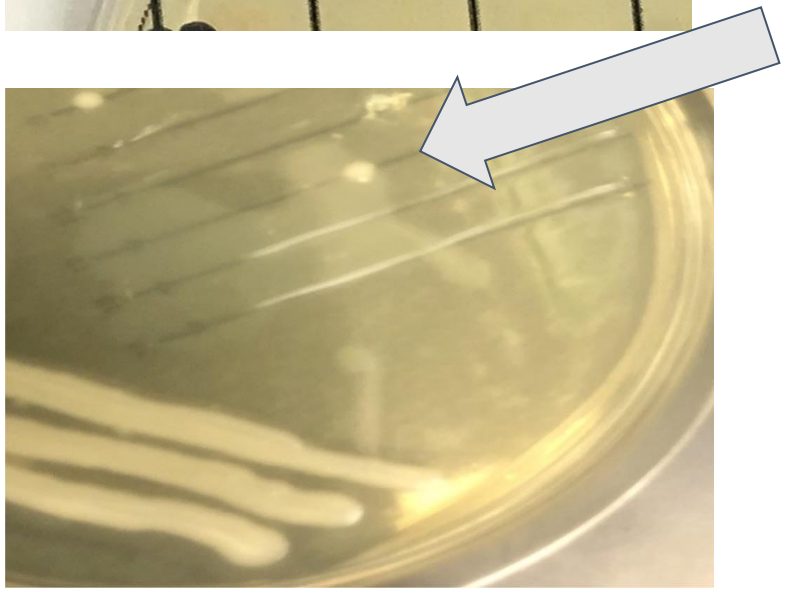


Anjali chose BHI as her other medium.

Labs 4 & 5

- **Lab 4 -** We chose two ESKAPE safe relatives to test our bacteria against. Then, we picked and put our bacteria on the safe relatives.

- **Lab 5 -** We checked to see if our bacteria had zones of inhibition. Afterwards, we streaked the ones that did for single colonies.



Antibiotic Activity Chart

Student	Own Soil	GPS Coordinates	CFU/g	Gram Negative (-) or Positive (+)
Meghan	Yes	26.36825” N 80.10400”W	2.8x10^7 CFU/G	Gram -
Angelica	No	26 degrees 22'10' N 80 degrees 6'12" W	9.2x10^8 CFU/G	Gram +
Anjali	Yes	26.19’26”N 80.4’58”W	2.0x10^9 CFU/G	Gram -

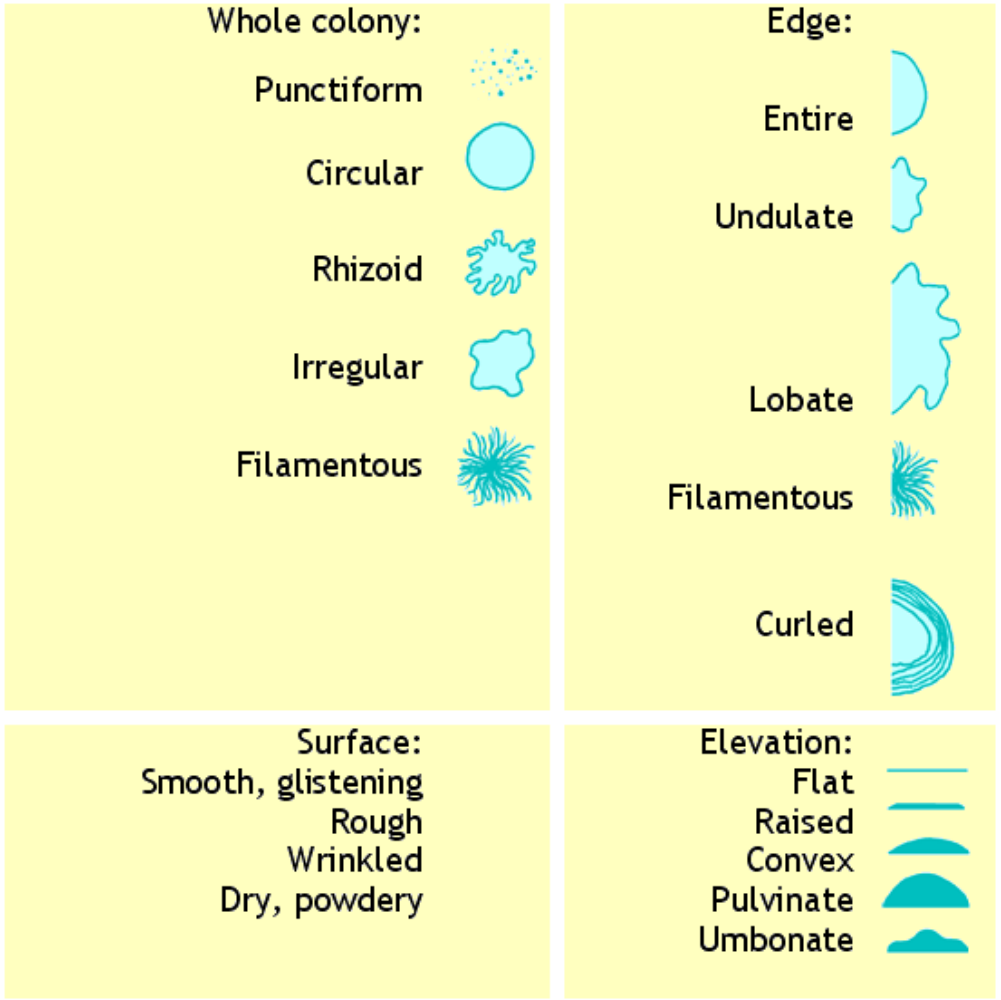
Antibiotic Activity Chart

Student	Medium	Temperature (c)	Light/Dark	Antibiotic Activity Against
Meghan	LB	30	Dark	*Bacillus subtilis*
Angelica	BHI	25	Dark	*Pseudomonas putida*
Anjali	BHI	30	Dark	*Escherichia coli*

Labs 6 & 7

Lab 6 - Determined colony morphology. Then, we streaked for single colonies and patched onto safe relative plates.

Lab 7 - Checked to see if our bacteria killed the safe relatives again. Then, we streaked for singles. Meghan and Anjali used their own soil while Angelica switched to someone else's.

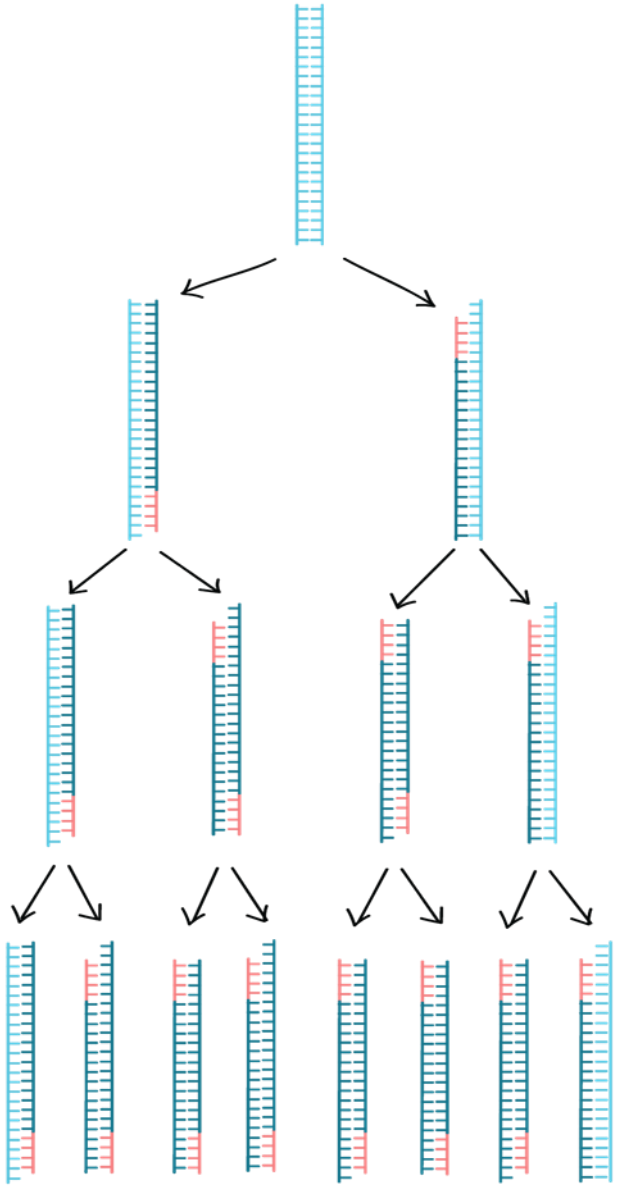


Source: commons.wikimedia.org

Labs 8 & 9

Lab 8 - PCR tubes, streaking, and swabbing.

Lab 9- Agarose gel electrophoresis and organic extraction.



Source:https://ka-perseus-images.s3.amazonaws.com/41f0e0fd8b49ba824db0eb707015557bb72ae72b.png

Lab 10

- MacConkey Agar
  - Streak for singles.
  - Angelica's did not grow (Gram positive).
  - Anjali and Meghan's grew (Gram negative and ferments lactose).

- SIM tube
  - See if our bacteria were motile.
  - None of them were.

- Catalase Test
  - Spread colony on clear slide.
  - Add drop of 3% H2O2.
  - Bubbles=catalase.
  - Meghan and Anjali's bacteria made catalase.
  - Angelica's bacteria didn't.

Angelica's bacteria did not grow, meaning it was Gram positive

Lab 10 Continued

- Next, we took our bottle of agar from Lab 9 and took a pipette to remove the top layer over the agar to put it into a small vial.

- Gram staining
  - Put 5ul off water on a slide.
  - Smeared a colony in the center of the water drop.
  - Heated the slide over a heat plate to dry.
  - Stained the plate crystal violet and safranin, used gram iodine, and 95% alcohol before stains.

- Viewed our slides under a microscope
  - Meghan and Anjali's slides were pink (Gram negative).
  - Angelica's slides were purple (Gram positive).

Labs 11 & 12

Lab 11 -

- Test isolates against all nine ESKAPE safe relatives and analyze antibiotic resistance.
- Making glycerol stock of our isolates.
- Testing organic extract against two safe relatives
- Examine SIM & add Kovac’s reagent
- Examine MacConkey plate

Lab 12 - Plug our bacteria’s DNA sequence data into the computer.

TABLE 2. Antibiotic Activity and Resistance of Your Bacterial Patches.

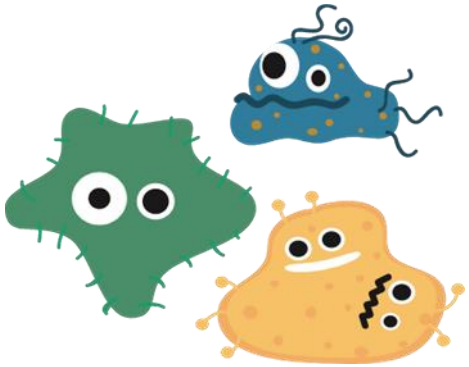
PLATE	Isolate	Lab	STUDENT	Kills S. epi	Kills E. coli	Kills E. caro	Kills E. raff	Kills A. bay	Kills B. sub	Kills E. aero	Kills M. smeg	Kills P. put	Res to Gram	Res to Tri	Res to Rif	Res to Tet	Res to P/S
BHI 30	AJE8	11am	Anjali Etwaru										Y	Y	Y	Y	Y
	QTWJR 19	11am	Angelica Prado- Diaz											Y			
	MVM1 4	11am	Meghan McCarthy			Y			Y			Y	Y	Y	Y	Y	Y

Organic Extract Results

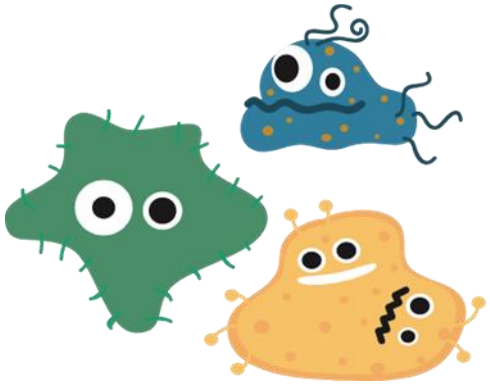
ISOLATE NAME	LAB	STUDENT'S NAME	Kills S. epi	Kills E. coli	Kills E. caro	Kills E. raff	Kills A. bay	Kills B. sub	Kills E. aero	Kills M. smeg	Kills P. put
AJE8	11am	Anjali Etwaru						Y			
MVM14	11am	Meghan McCarthy						Y			
QTWJR19	11am	Angelica Prado- Diaz						Y			

DNA Sequence Results

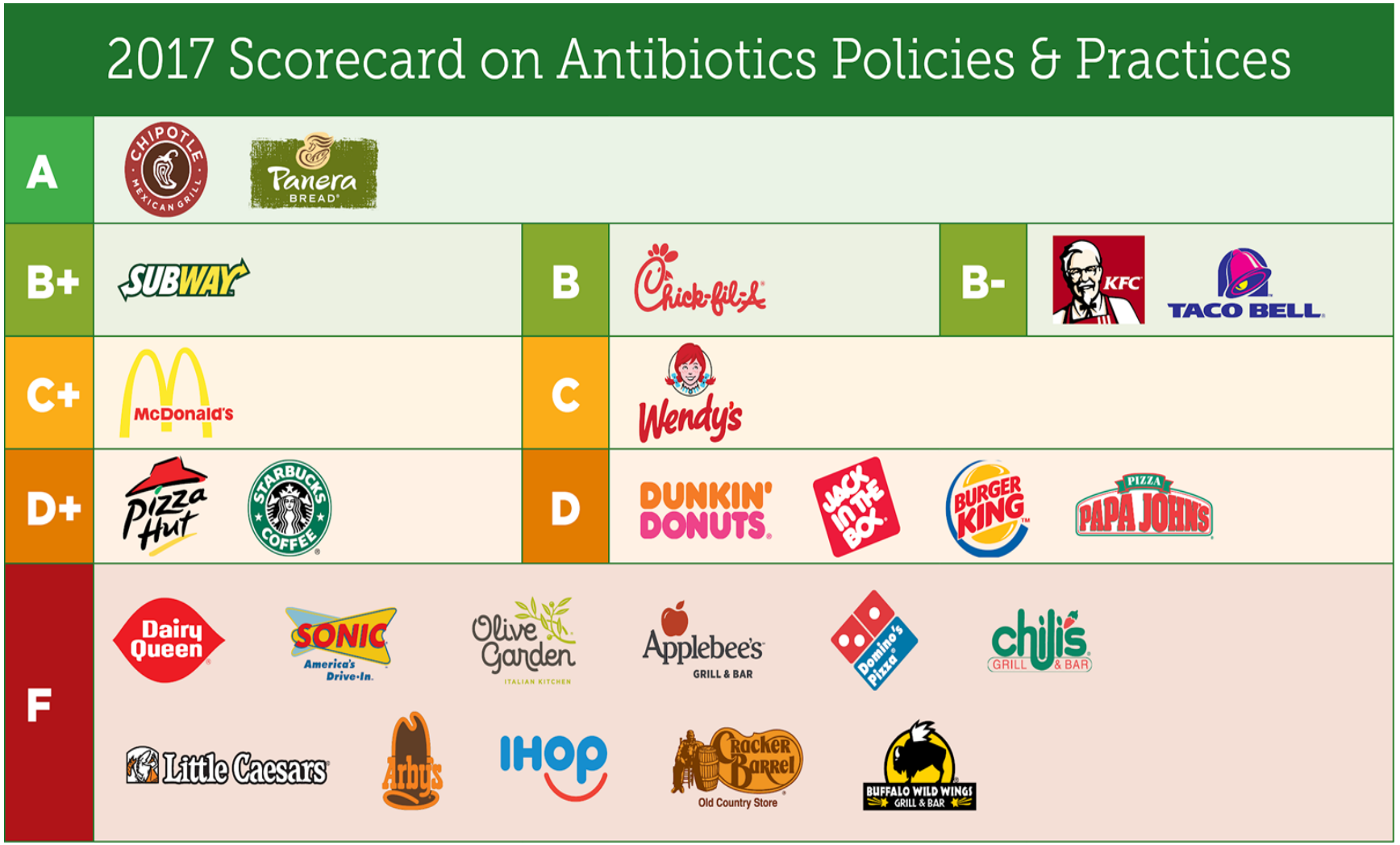
	Angelica	Anjali	Meghan
PCR Product and Sequence	No	Yes	No
Organism with closest match to DNA sequence	*Bacillus subtilis* strain SCODB99 16S ribosomal RNA gene, partial sequence.	*Pseudomonas putida* strain SG 5 16S ribosomal RNA gene	*Pseudomonas baetica* strain Hb-10 16S ribosomal RNA gene
Percent identity	99%	98%	99%



DISCUSSION



- Feeding animals antibiotics is a major contributing factor to the crisis.
- How can we encourage/pressure companies to stop this action?



Source: https://foe.org/resources/chain-reaction-iii-report/

Potential Future

This class has given us as students and scientists an insight on how severe the antibiotic crisis really is. It has also taught us many efficient lab techniques and proper lab safety.

Science is not about being right every time. Usually it takes hundreds of attempts just to find a solution.

Although our antibiotics may not work the way we wanted, our information and findings can help future scientists and SWI programs inch closer to ending the antibiotic crisis. Perhaps our bacteria can be tested against ESKAPE pathogens.

Illustration by Paul Blow
http://stanmed.stanford.edu/2015summer/upfront/a-safer-antibiotic.html