Beyond the Burn

The Secret World of UTIs

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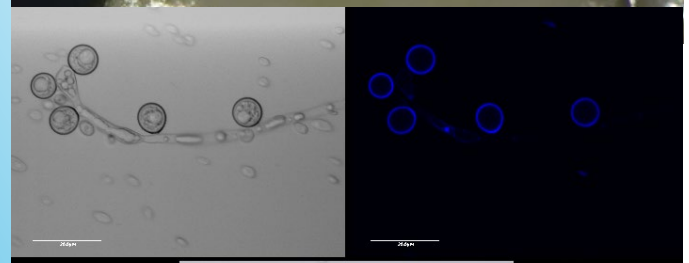
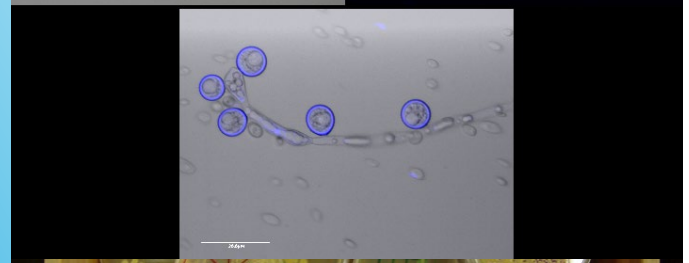
20 April 2022





Disclaimers

I am employed by CirrusDx

- We conduct UTI testing (and other tests)

I am in love with microbiology

- That is my perspective… I leave the hard stuff to you all!

NOT my actual drawer at work!!

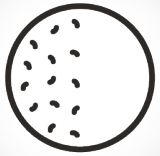
A Journey Through the World of UTIs

UTI Basics:
What you need to know about UTIs

Into the Unknown:
Making sense of all the testing options

Finish!






Secret Life of P:
A quiz about UTIs

New and Exciting:
What's new in the world of UTIs?

Two Roads:
A Patient Case Study

The Secret Life of P

A quiz about UTIs

The Secret Life of P

1. Grab a set of answer cards (labeled A, B, C, D).
2. Read the question on screen.
3. Answer the question on screen by holding up the appropriate card.
4. See the correct answer (then bask in the glory of victory or wallow in the misery of defeat, as applicable.)

Question 1

True or False: The bladder is sterile.

A. True

B. False

Question 1

True or False: The bladder is sterile.

A. True

B. False

Answer: B, False

Research shows a bladder microbiome. Bacteria can be found in urine samples from both symptomatic and asymptomatic patients.

Question 2

True or False: Among younger adults (20 - 50 years old), UTIs are more common in men.

A. True

B. False

Question 2

True or False: Among younger adults (20 - 50 years old), UTIs are more common in men.

A. True

B. False

Answer: B, False

UTIs are 50 times more common in women in this age group than men! It starts to “even out” as people age.

Question 3

The term “mellitus” (as in diabetes mellitus) is translated as “flavored with honey” in Latin for what reason?

A. Urine was tasted to diagnose certain conditions

B. Urine was mixed with honey to diagnose certain conditions

C. Urine color and texture were compared to honey to diagnose certain conditions

Question 3

The term “mellitus” (as in diabetes mellitus) is translated as “flavored with honey” in Latin for what reason?

A. Urine w

B. Urine w

C. Urine c … se certain co

Answer: A

In ancient times, some diseases (like diabetes and even UTIs!!!) were discovered and diagnosed by tasting a patient’s urine. Pathologists were trained in tasting urine up until the 1940s. Aren’t you grateful for modern testing?

Question 4

_____ is defined as a thin, usually resistant layer of microorganisms (such as bacteria) that form on and coat various surfaces.

A. Biolining

B. Pus

C. Biofilm

D. Microbiome

Question 4

_____ is defined as a thin, usually resistant layer of microorganisms (such as bacteria) that form on and coat various surfaces.

A. Biolining

B. Pus

C. Biofilm

D. Microbic

Answer: C, Biofilm

Biofilm is defined as a thin, usually resistant layer of microorganisms (such as bacteria) that form on and coat various surfaces.

Question 5

What does AST stand for (in the context of UTI testing)?

A. Antimicrobial Symptomatic Technology

B. Alternate Symptomatic Transferase

C. Automatic Syndromic Testing

D. Antimicrobial Susceptibility Testing

Question 5

What does AST stand for (in the context of UTI testing)?

A. Antimicrobial Symptomatic Technology

B. Alternate Symptomatic Transferase

C. Automatic Syndromic Testing

D. Antimicrobial Susceptibility Testing

Answer: D, Antimicrobial Susceptibility Testing (AST)

A procedure used to determine which antibiotics a specific organism or group of organisms are susceptible to.

Question 6

Biofilms can make organisms how much more resistant to antibiotics than organisms not in a biofilm?

A. 100x

B. 500x

C. 1000x

D. 5000x

Question 6

Biofilms can make organisms how much more resistant to antibiotics than organisms not in a biofilm?

A.

B.

C.

D.

Answer: C, 1000 times more resistant

- Antibiotics have a difficult time getting through the biofilm matrix
- Resistance genes can be transferred between organisms in biofilms
- pH in a biofilm can be different than outside… and can inactivate antibiotics
- Not all cells in a biofilm are metabolically active… many antibiotics need actively growing bacteria to work…no bacterial "zombies"!!

Question 7

Which of the following is the most common cause of UTIs?

A. Klebsiella pneumoniae

B. Candida albicans

C. Proteus mirabilis

D. Escherichia coli

Question 7

Which of the following is the most common cause of UTIs?

A. Klebs

B. Cand

C. Prote

D. Esch

Answer: D, *Escherichia coli* (*E. coli*)

E. coli, *Proteus*, *Enterococcus* , *Pseudomonas*, *Enterobacter* , *Serratia*, and *Candida* are all common causes of UTIs.

Question 8

When grown on a plate, which organism is said to smell like grapes?

A. Proteus mirabilis

B. Pseudomonas aeruginosa

C. Escherichia coli

D. Candida albicans

Question 8

When grown on a plate, which organism is said to smell like grapes?

A. Proteus mirabilis

B. Ps

C. Es

D. Ca

Answer: B, *Pseudomonas aeruginosa*

Note: don't inhale. Just whiff.
Proteus is said to smell like German Chocolate Cake.
Candida is said to smell like bread.
It all comes back to food!!

Question 9

True or False: Standard culture is able to detect/grow all uropathogens.

A. True

B. False

Question 9

True or False: Standard culture is able to detect/grow all uropathogens.

A. Tru

B. Fa

Answer: B, False

Some uropathogens prefer different growth conditions than what is standardly performed.

Question 10

Which statement is true about PCR testing?

A. It can detect living and dead organisms.
B. It is a method that is too sensitive for use in diagnostics.
C. It takes several days to run this test for UTIs.
D. It is the most common method for UTI testing.

Question 10

Which statement is true about PCR testing?

A. **Answer: A**

B. **It can detect living and dead organisms.**

C. It takes several days to run this test for UTIs.

D. It is the most common method for UTI testing.

Question 11

Which statement is true about next generation sequencing (NGS)?

A. It is the fastest method for detecting pathogens.
B. If a resistance gene is detected, then the organisms are resistant to that drug.
C. If a resistance gene is not detected, then the organisms are susceptible to that drug.
D. It is capable of determining the entire resistome of an organism.

Question 11

Which statement is true about next generation sequencing (NGS)?

Answer: D

It is capable of determining the entire resistome of an organism.

A.

B.

C. If a resistance gene is not detected, then the organisms are susceptible to that drug.

D. It is capable of determining the entire resistome of an organism.

Question 12

Why is antimicrobial stewardship important?

A. To preserve antimicrobials for use in complicated and serious infections.
B. To preserve antimicrobials for future generations to use.
C. To make sure additional harm is not done to the patient.
D. To keep the native microbiome intact as much as possible.

Question 12

Why is antimicrobial stewardship important?

A. T
a

B. T
u

to

Answer: Trick question!

ALL of these statements are true!

C. To make sure additional harm is not done to the patient.

D. To keep the native microbiome intact as much as possible.

Question 13

What percentage of sepsis cases originate from the urogenital tract?

A. 10%

B. 25%

C. 50%

D. 75%

Question 13

What percentage of sepsis cases originate from the urogenital tract?

A. 10%

B. 25%

C. 50%

D. 75%

Answer: B, About 25% cases originate in the urogenital tract.

Question 14

What is the approximate percentage of UTIs caused by *E. coli*?

A. 15-25%

B. 30-45%

C. 50-60%

D. 80-90%

Question 14

What is the approximate percentage of UTIs caused by *E. coli*?

Answer: D

Approximately 90% of UTIs are caused by *E.coli.**

*The numbers vary here, depending on what resources you use. **The big takeaway is that it's a LOT!**

Question 15

When determining susceptibilities phenotypically, what is happening in the lab?

A. The organisms are grown in the presence of the different drugs

B. PCR is used to detect resistance genes

C. Resistance genes are detected using next generation sequencing

Question 15

When determining susceptibilities phenotypically, what is happening in the lab?

A. T
dif

B. I

C. Resistance genes are detected using next generation sequencing

Answer: A

The organisms are grown in the presence of the different drugs.

Question 16

True or False: Genotypic assays have the ability to detect resistance but not susceptibility.

A. True

B. False

Question 16

True or False: Genotypic assays have the ability to detect resistance but not susceptibility.

A

B

Answer: A, True

Genotypic assays have the ability to detect resistance but not susceptibility.

Question 17

True or False: One of the powers of phenotypic testing is that it provides key information about mutations.

A. True

B. False

Question 17

True or False: One of the powers of phenotypic testing is that it provides key information about mutations.

A

B

Answer: B, False

Phenotypic testing's power is the ability to provide information about susceptibility.

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UTI Basics

Everything you ever wanted to know about UTIs (and some stuff you didn’t)

P Quiz!

In long-term care settings, where do UTIs rank as far as prevalence?

A. The MOST common infection

B. The second most common infection

C. The third most common infection

P Quiz!

In long-term care settings, where do UTIs rank as far as prevalence?

Answer: B, Second most common infection

UTIs are the second most common infection in LTC settings, with a prevalence of 0.6% - 21.8%.

UTI Stats

150 million people affected worldwide

In 2007:

- 10.5 million office visits (0.9% of all ambulatory visits)
- 2-3 million ED visits

Estimated cost: $3.5 billion/year

In Long Term Care Setting:

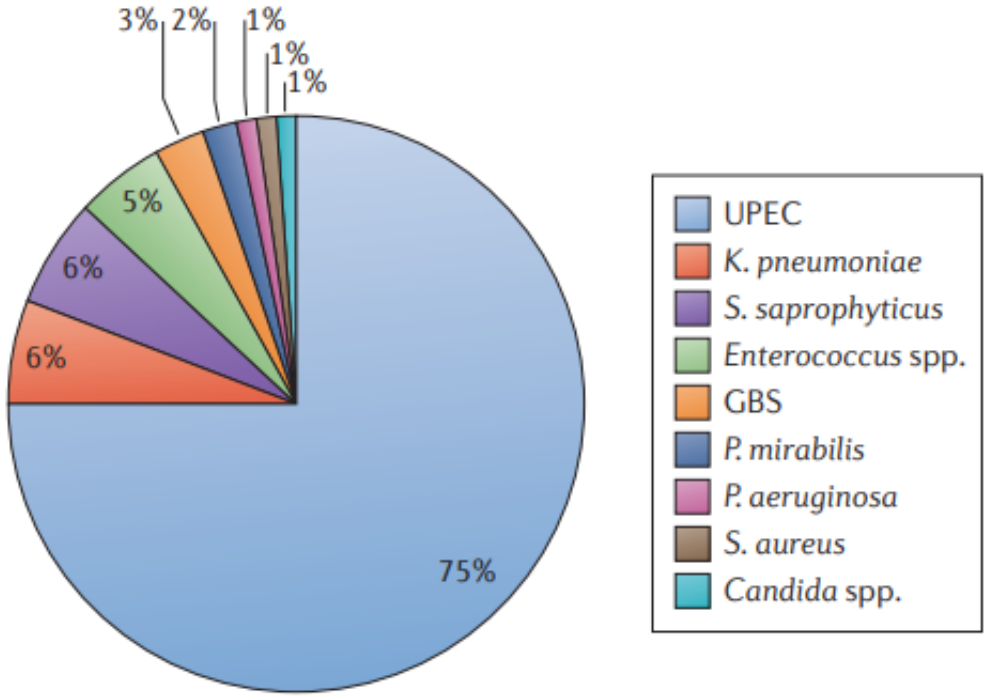
- 2nd most frequent infection
- Prevalence: 0.6%- 21.8%
- Treatment accounts for 30 -50% of antibiotic use
- High numbers of asymptomatic bacteriuria: 18 -57% in women; 19-38% in men (non-catheterized)
- Risk of UTIs in catheterized patients is time dependent, increasing 3-8% each day (100% prevalence at 30 days)

Multi Drug Resistance (MDR) is a problem in LTC residents

- Study of LTC residents in ED for UTI: MDR bacteria in 39-80% of bacteria isolated

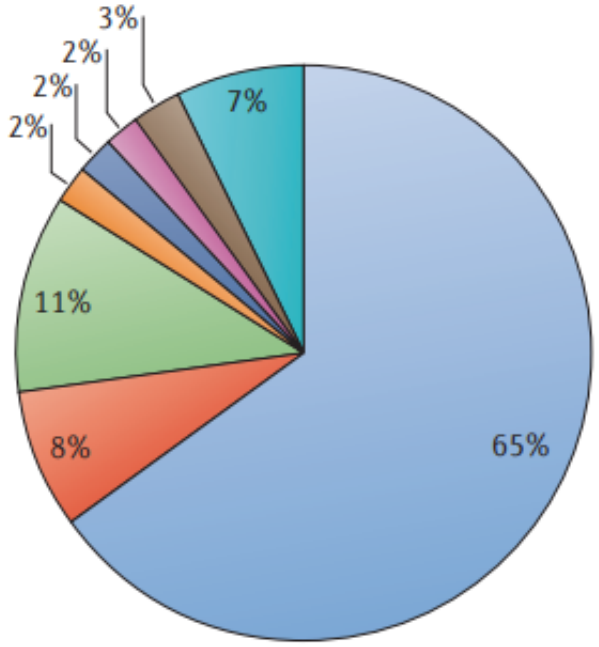
The Epidemiology of UTIs

Uncomplicated UTI



Risk factors
- Female gender
- Older age
- Younger age

Complicated UTI



Risk factors
- Indwelling catheters
- Immunosuppression
- Urinary tract abnormalities
- Antibiotic exposure

Urosepsis

Overall population: 25 % sepsis cases originate from urogenital tract

In US, last 30-40 years sepsis cases increasing

- Mortality from sepsis decreasing
- Estimated mortality fro m urosepsis: 30-40%

UTI is most common cause of nursing home acquired bacteremia (51-56%) and hospitalizations

- Lower mortality than pneumonia (NY study: mortality 8% vs 56%)

Up to 33% mortality rate in elderly patients where UTI caused bacteremia

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New and Exciting

New concepts in the world of UTIs

P Quiz!

What percentage of UTIs are caused by polymicrobial infections?

A. Approximately 30%

B. Approximately 20%

C. Approximately 50%

P Quiz!

What percentage of UTIs are caused by polymicrobial infections?

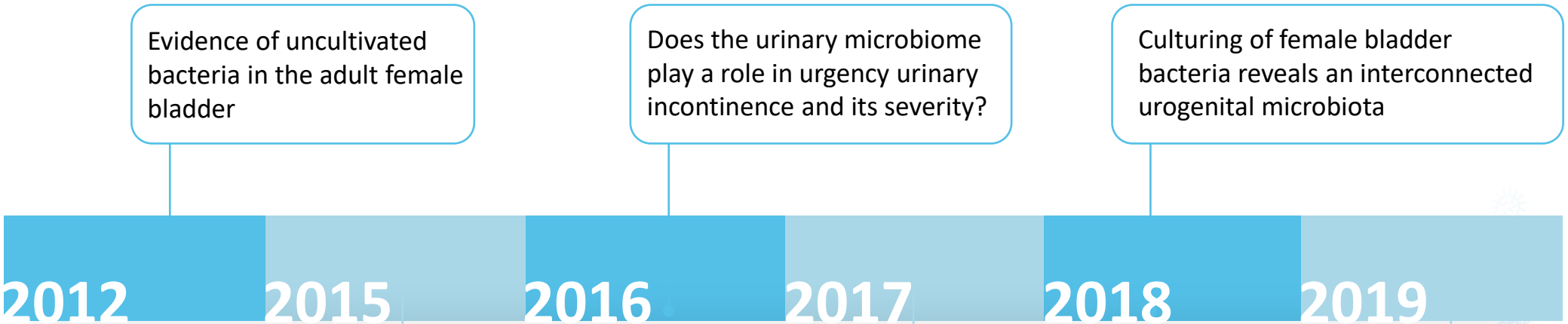
A. Approximately 30%

Answer: A

Approximately 30% (one-third) of UTIs are caused by polymicrobial infections!

New Data: Is the Bladder Really Sterile?

Accumulating body of evidence says " The bladder is **NOT** sterile"



2012 — Evidence of uncultivated bacteria in the adult female bladder

2015
- The bladder is not sterile: history and current discoveries on the urinary microbiome
- "Sterile urine" and the presence of bacteria

2016 — Does the urinary microbiome play a role in urgency urinary incontinence and its severity?

2017 — 'Urinary tract infection' and the microbiome

2018 — Culturing of female bladder bacteria reveals an interconnected urogenital microbiota

2019
- Urinary fungi associated with urinary symptom severity among women with interstitial cystitis/bladder pain syndrome
- The bladder is not sterile: an update on the urinary microbiome

*This is a small sampling of the body of literature addressing this topic

So the Bladder isn't Sterile: Who Cares?

Some bacteria may always be there

- Maybe have some sort of protective purpose?

Older we get, more likely finding bacteria without symptoms

We don't yet know what it all means…

- Who is in and who is an invader if there is a urinary biome?

New Concept: More Than One Organism Can Cause a UTI

Polymicrobial infections = 2 or more organisms

- *E. coli* with *Proteus*
- *E. coli* with *Enterococci*
- *Enterococci* with *Proteus*
- *Klebsiella* with *E. coli*

30-40% of UTIs could be polymicrobial

Increased pathogenic potential

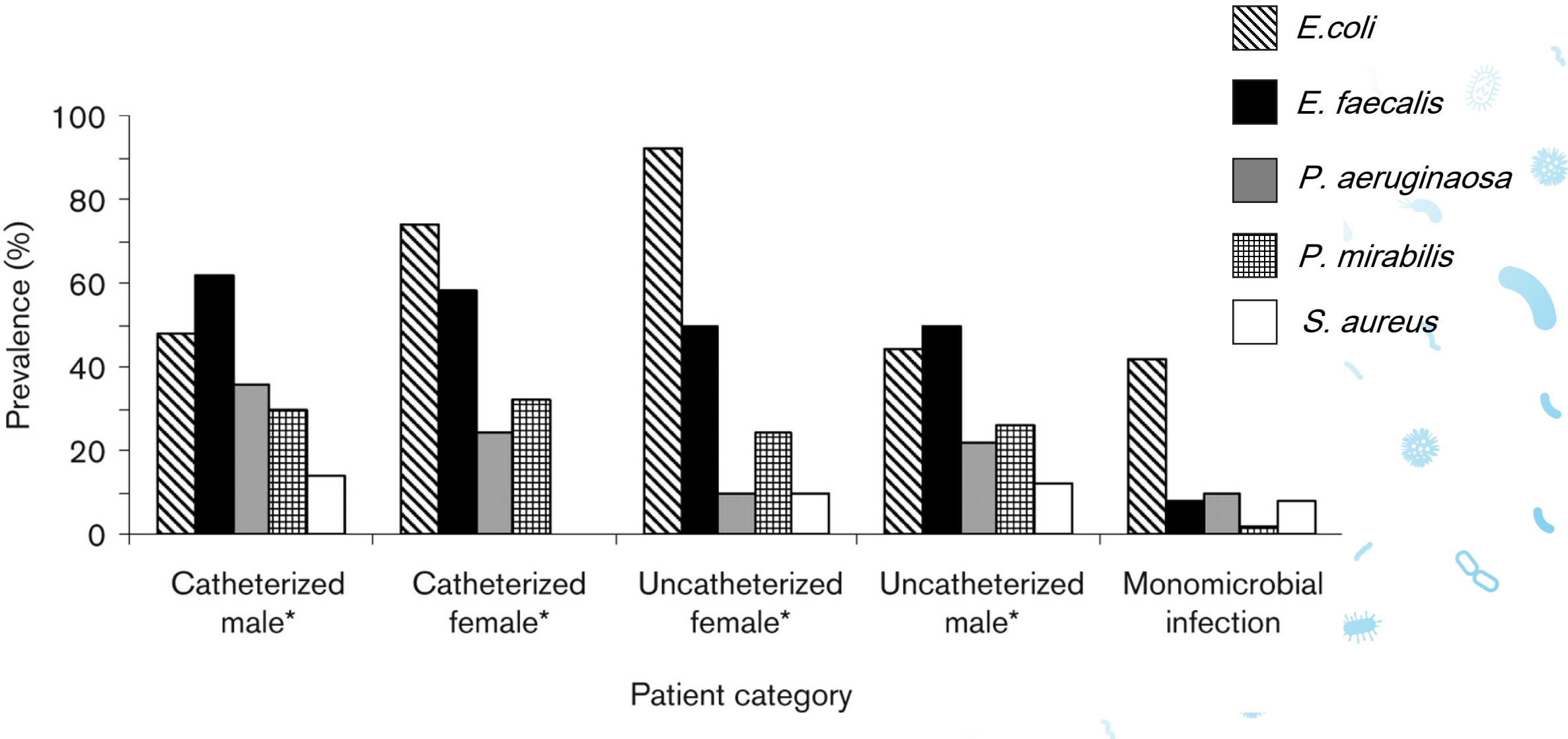
In Long Term Care:

- 10-20% non catheterized patients have polymicrobial UTI
- Almost 100% catheterized patients have polymicrobial UTI

E. coli and *Enterococcus* common together in older women and those with frequent/recurrent UTI

- Significance not yet known

Distribution of Pathogens in Polymicrobial Samples



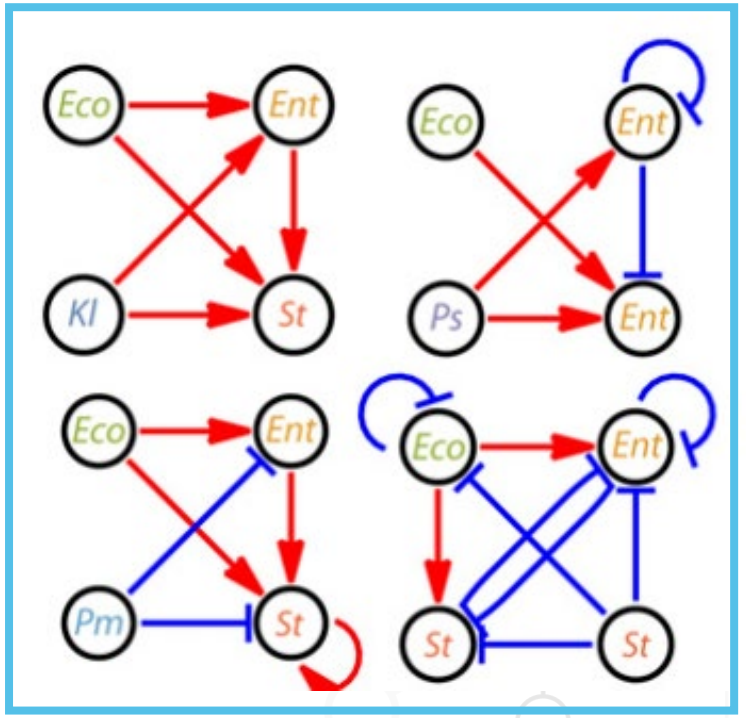
Polymicrobial Interactions

Red = positive interactions
Blue = negative interactions

When *E. coli* is present in polymicrobial UTI samples, it is statistically more invasive than *E. coli* alone

- Establish reservoirs (intracellular communities) that offer some level of protection
- Also more resistant to ciprofloxacin and trimethoprim than *E. coli* from monomicrobial cultures
    - Note: organisms were isolated before testing susceptibilities

Presence of *Enterococcus faecalis* might make virulent *E. coli* more pathogenic



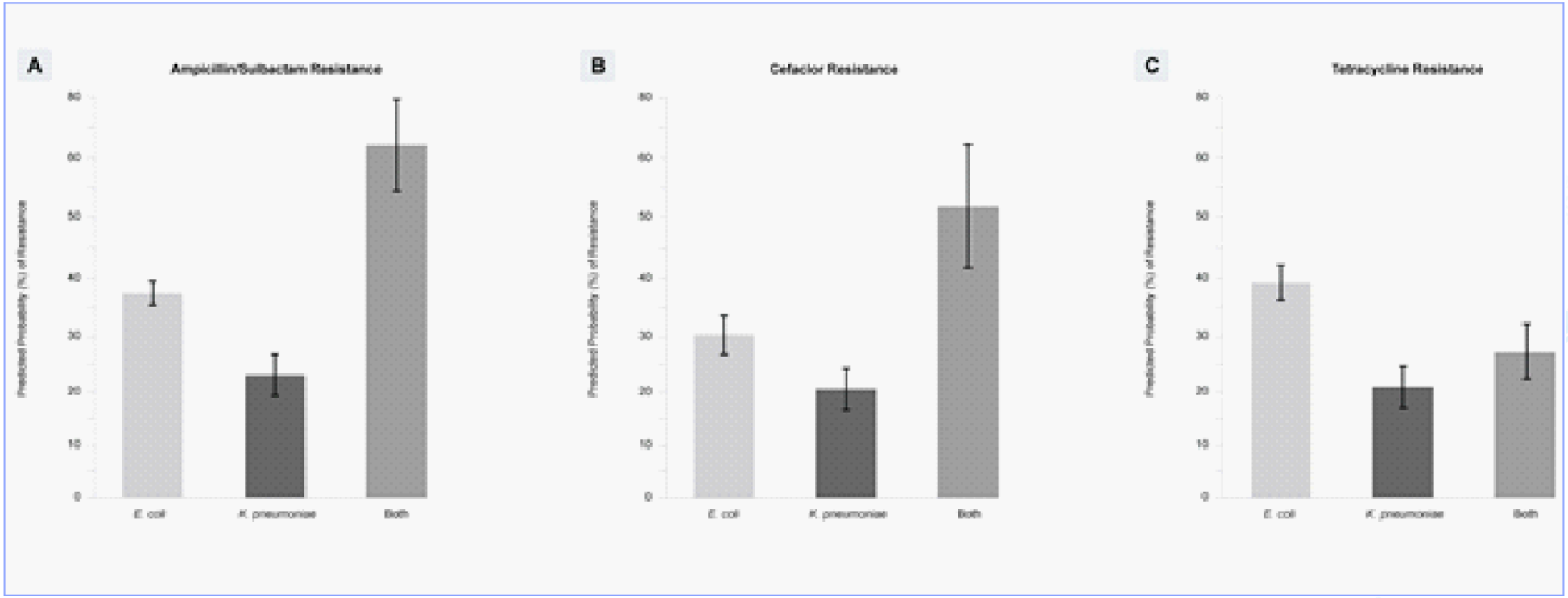
Eco = *E. coli*
Ent = *Enterococcus* species (*E. faecalis*, *E. faecium*)
Kl = *Klebsiella* species (*K. pneumoniae*, *K. oxytoca*)
Pm = *Proteus mirabilis*
St = *Staphylococcus* species (*S. aureus*, *S. haemolyticus*, *S. capitis*)

Polymicrobial Interactions Can Influence Resistance Patterns

Ampicillin/Sulbactam Resistance

Ceflacor Resistance

Tetracycline Resistance

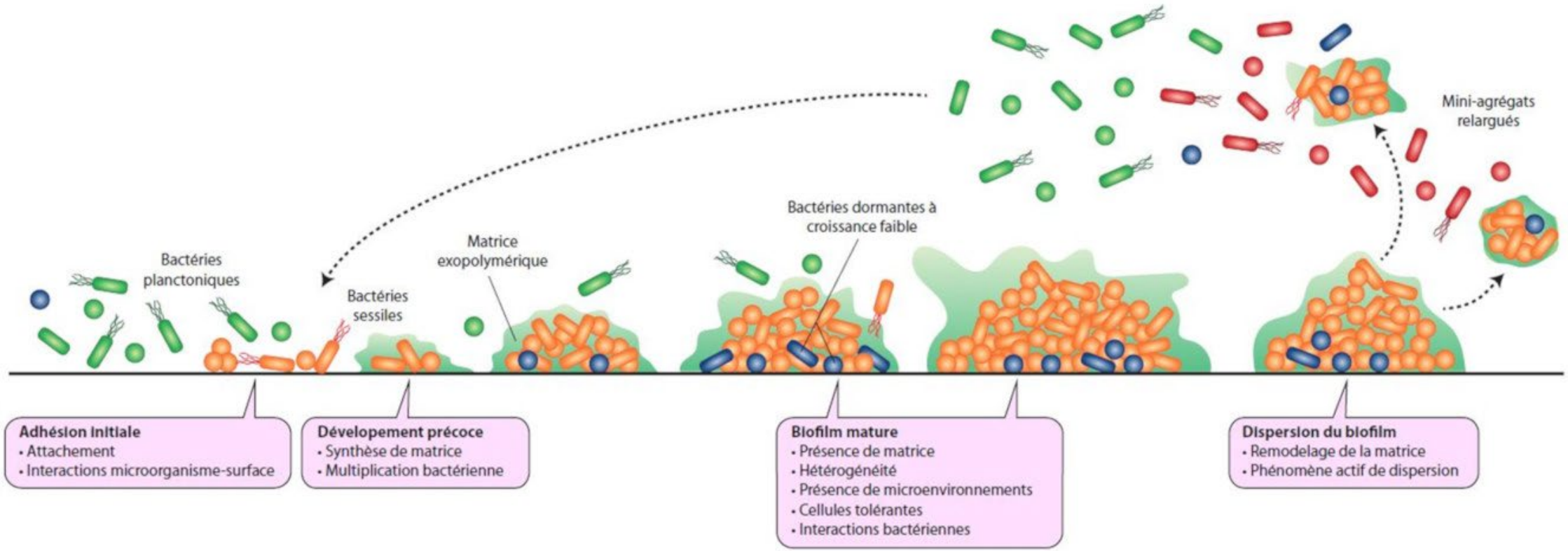


E.coli

K. pneumoniae

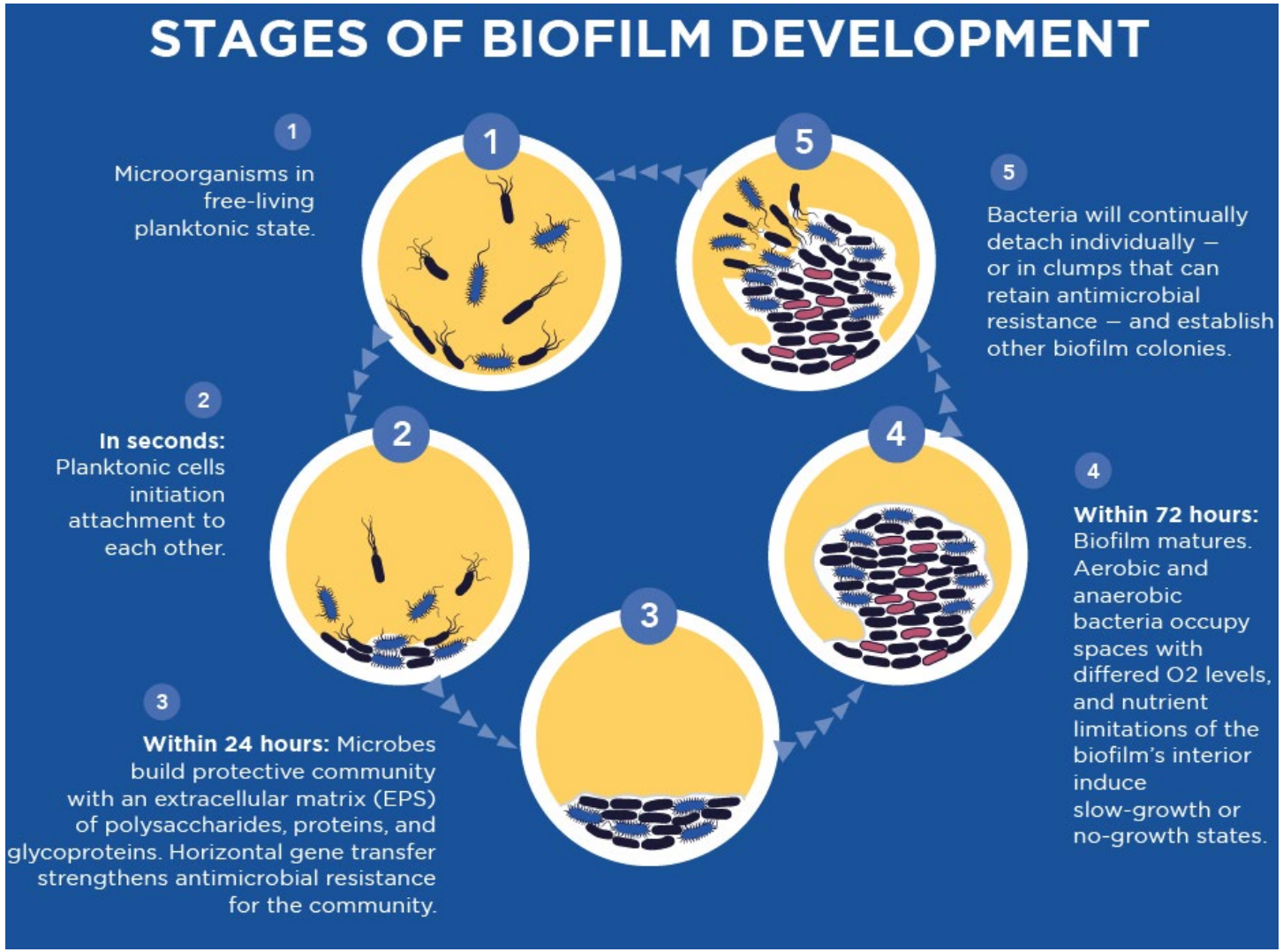
Both

“Old” Concept, Fresh Look: Biofilms



Can be monomicrobial; *E. coli* is known to form biofilms on its own
Does not have to be associated with a catheter

Biofilm Development: An English Version



Biofilm Most Wanted

Commonly studied in catheter -associated UTIs

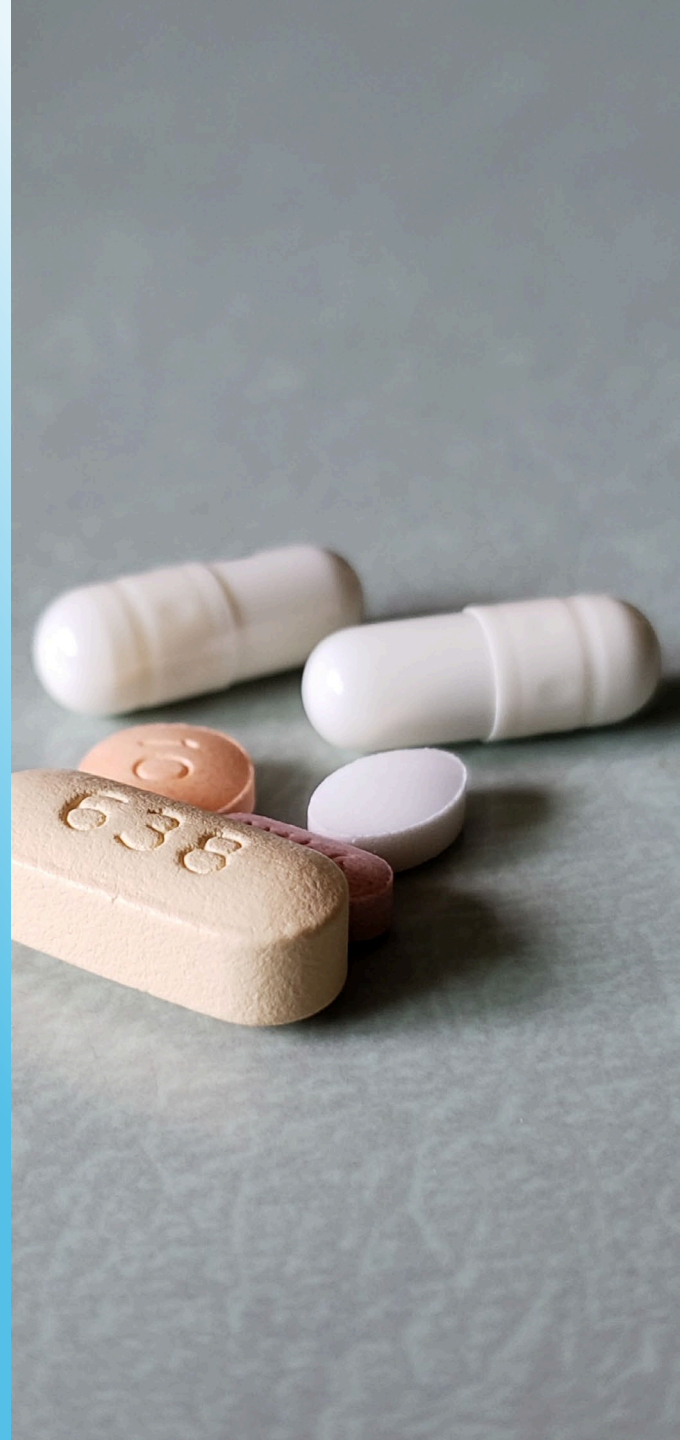
- But evidence building that not just catheters

Most common pathogens: *E. coli*, *Proteus*, *Enterococcus* , *Pseudomonas*, *Enterobacter* , *Serratia*, and *Candida*

- Many of these bacteria are particularly “good” at biofilm formation; several virulence factors present that contribute

Biofilms can make organisms 1000 -fold more resistant to antibiotics than organisms not in a biofilm

- Antibiotics have a difficult time getting through the biofilm matrix
- Resistance genes can be transferred between organisms in biofilms
- pH in a biofilm can be different than outside… and can inactivate antibiotics
- Not all cells in a biofilm are metabolically active… many antibiotics need actively growing bacteria to work



UTI Burden on Antimicrobial Stewardship

Antibiotic use for UTIs #2 behind Respiratory tract infections

Increase in MDR (multi-drug resistant) organisms causing UTIs in past few decades

- Treatment more complicated
- Thought to stem from overuse of antibiotics
  - Suspected UTIs treated
  - Results typically take 2-3+ days

Stewardship Thoughts

If the bladder is not sterile, are we doing more harm by using antibiotics?

- Disrupting the urinary microbiome, the gut microbiome, etc.

Which is more harmful in each patient's case? Using antibiotics or not using antibiotics?

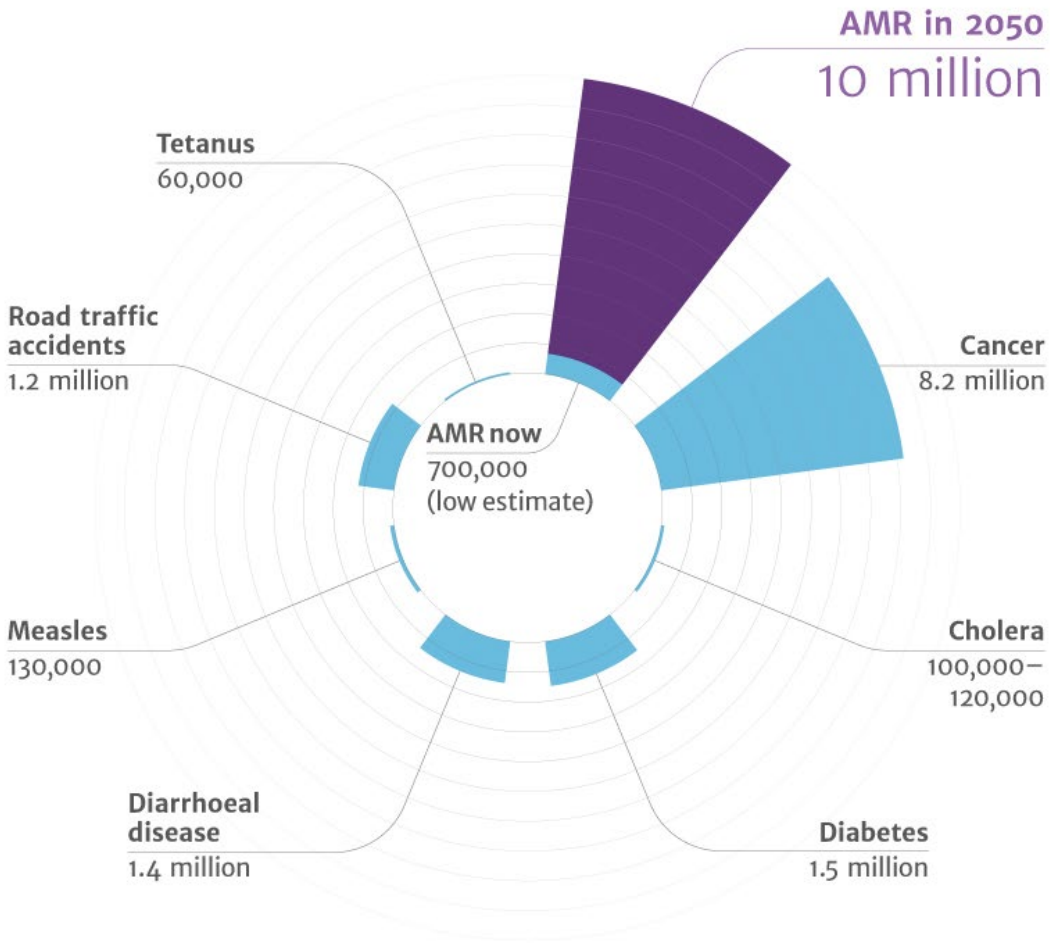
And what about the virome (viruses of the microbiome)?!?

Lots of antibiotic treatment over time = altered flora, increase in resistance, *C. difficile*

Using antibiotics can leave available niches no longer filled by altered microbiota and allow for uropathogens to enter and colonize

Stewardship = protectors of the microbiome

Why Stewardship Matters



UTIs Rank 4th in Global Deaths Associated with and Attributed to Resistance

Bone+ = infections of bones, joints, and related organs

BSI = bloodstream infections
Cardiac = endocarditis and other cardiac infections

CNS = meningitis and other bacterial CNS infections

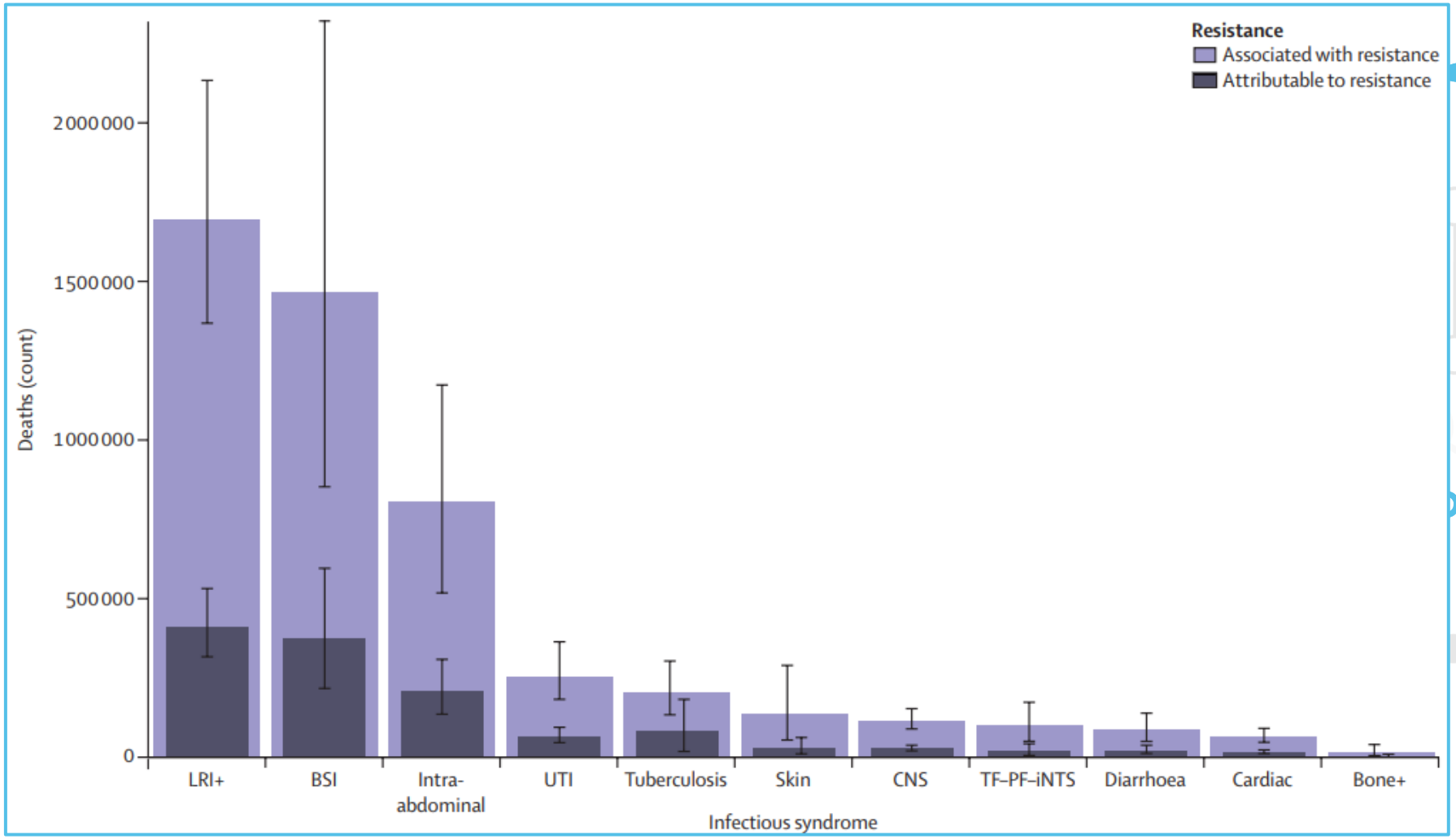
Intra-abdominal = peritoneal and intra-abdominal infections

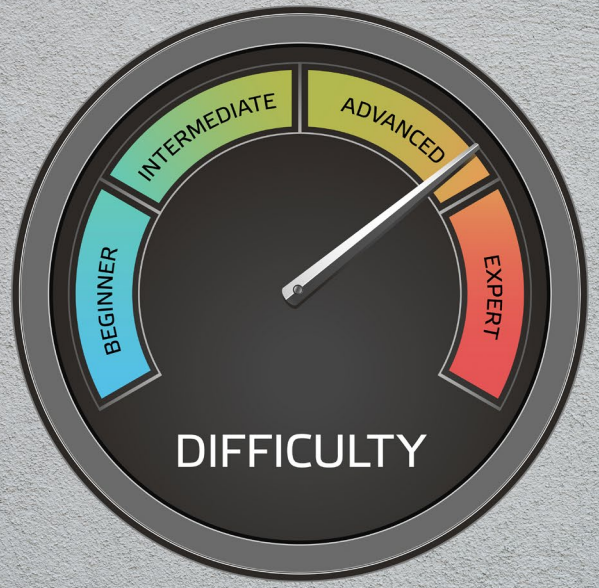
LRI+ = lower respiratory infections and all related infections in the thorax

Skin = bacterial infections of the skin and subcutaneous systems

TF–PF–iNTS = typhoid fever, paratyphoid fever, and invasive non-typhoidal *Salmonella* spp

UTI = urinary tract infections and pyelonephritis.





Diagnosing UTIs Is Not Easy

A whole session could be dedicated to who to test and when to test…

Why so complicated?!?

- Many studies do not include males and the elderly

What if the bladder isn't sterile?

- What does "significant bacteruria" then mean

How helpful is the phrase "symptoms referrable to the urinary tract" when trying to decide if a UTI is present?

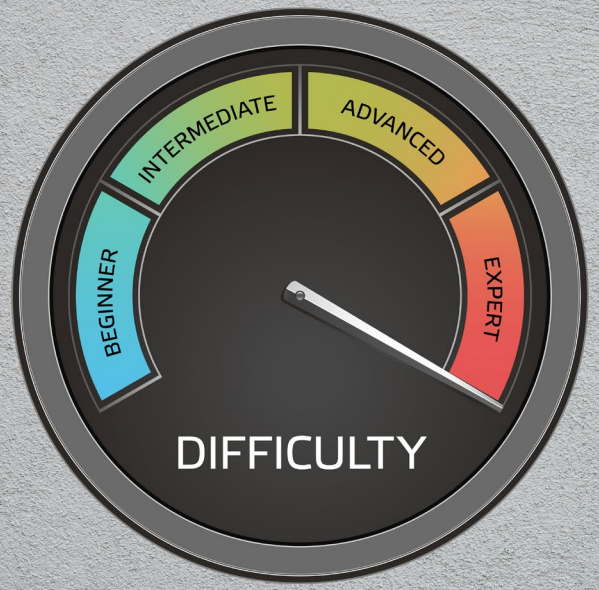
I'm sorry… the lab cannot (yet) distinguish between infection and colonization

- Lab provides information
- Clinician/Lab partnership needed in many instances to figure out some of the tough cases

Today's goal: learn how the tests you order affect what you do

Recommended reading: "Urinary Tract Infection" – Requiem for a Heavyweight

- Finucane TE. "Urinary Tract Infection"-Requiem for a Heavyweight. *J Am Geriatr Soc*. 2017;65(8):1650-1655. doi:10.1111/jgs.14907



To Make It More Difficult…

Symptoms + “significant” bacteriuria = symptomatic UTI

NO agreement on evidenced based definition of symptoms in LTC residents

- Yet distinguishing asymptomatic from symptomatic is super important!!

No gold standard, in a sense

- Bacteriuria with pyuria is common in asymptomatic LTC residents

Testing it not the “magic bullet”

- Clinical judgement is needed too

The Great Push and Pull of Different Forces When Making the Decision to Test and/or Treat

Patient with “symptoms” and/or worried family members

Effects of using antibiotics (nothing is without risk) (disrupt normal microbiome, other side effects, *C. difficile*)

Risk of serious illness, such as sepsis

What about stewardship and antimicrobial resistance?

What if the “symptoms” are actually caused by something else and this is asymptomatic bacteria (now that we “know” the bladder is not sterile)

Patient history; co-morbidities

Acknowledgement: You Need Better Tools

We are so not there yet!!

- Consequences of wrong treatments: deteriorating patient health/discomfort, switching antibiotics, prolonged antibiotic exposure
- But for now, let's understand the tools that you do have and how to use them.

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Into the Unknown

A look at testing methods and how they work

P Quiz!

True or False: The sum of an organism's observable characteristics is known as its genotype.

A. True

B. False

P Quiz!

True or False: The sum of an organism's observable characteristics is known as its genotype.

Answer: B, False

Genotype refers to genetic material passed between generations, while phenotype is the sum of an organism's observable characteristics.

Defining Phenotype and Genotype

Definitions:

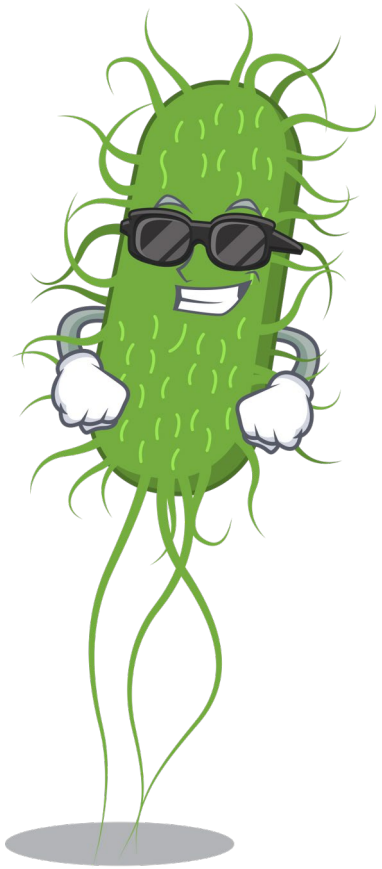
- Genotype: genetic makeup of the bacteria (what is in its DNA)
- Phenotype: the detectable expression of a trait

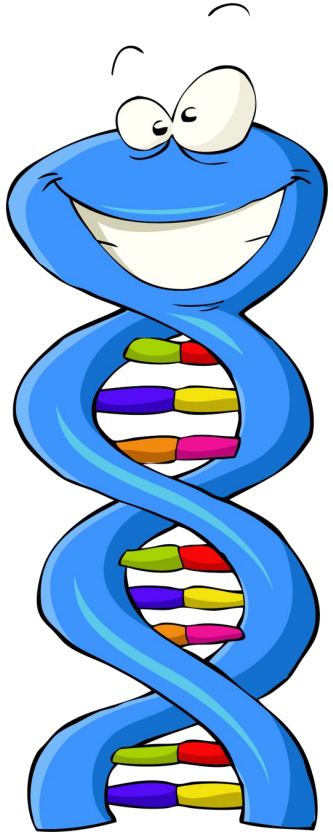
Applying these to susceptibility testing…

- Phenotypic resistance determination
  - I take organisms. I take antimicrobials. I put them together on/in super rich media. I let them interact and see what happens. This tells me exactly how these organisms actually respond to the antimicrobials that may be used to treat this infection.
  - “What you see is what you get”
- Genotypic resistance determination
  - I take organism. I use PCR to find known genetic markers (sequences in the DNA), or I use sequencing to find every potential genetic resistance marker(s) that has been cataloged in a database.

Phenotype and Genotype

What is on the outside vs what is on the inside

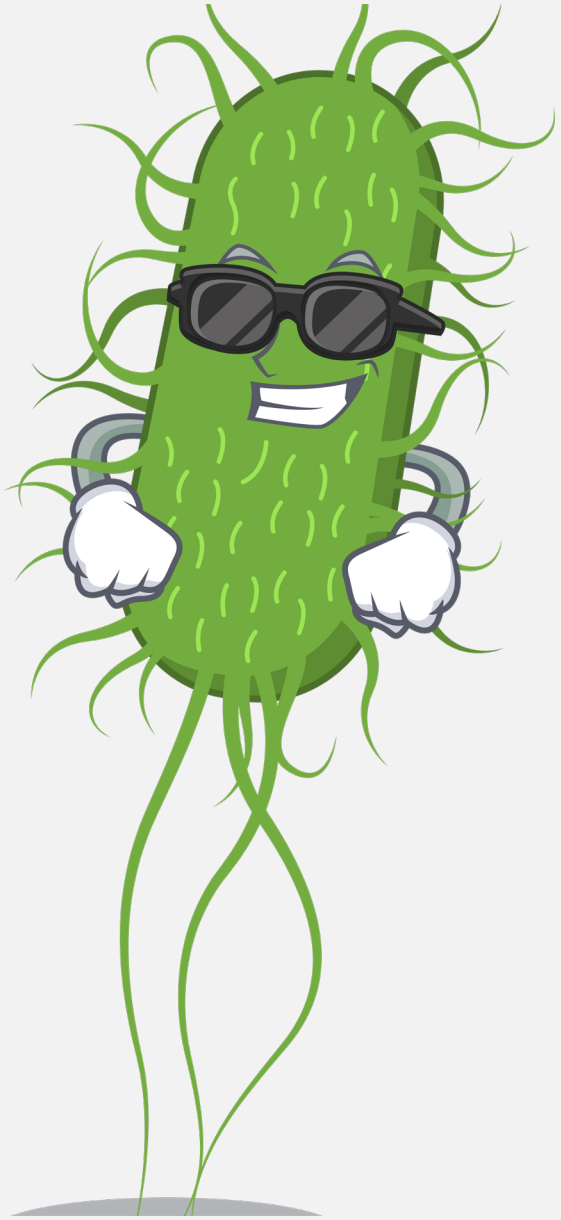




Relatable Example of Phenotype/Genotype First

The story of little “i” vs BIG “I”

Genotype	Red blood cell appearance	Phenotype (blood group)
I^AI^A or I^Ai		A
I^BI^B or I^Bi		B
I^AI^B		AB
ii		O



Method 1: “Traditional” UTI culture (SUC and EQUC) (Phenotypic)

SUC: Standard Urine Culture

EQUC: Expanded Quantitative Urine Culture

<u>Standard Urine Culture</u>

Jokingly referred to as the “ *E. coli* and friends detector”

- Friends: *Staphylococcus saprophyticus* , *Klebsiella pneumoniae* , *Proteus mirabilis*

Thresholds set arbitrarily… in 1950s…colony count isn’t linked to who might become more ill, septic, who might recover, who needs treatment

<u>Expanded Quantitative Urine Culture</u>

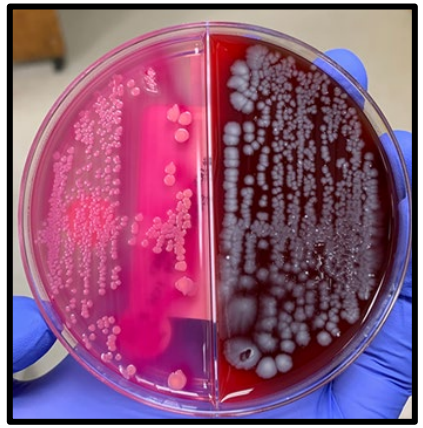
Moving beyond just “ *E. coli* and friends”

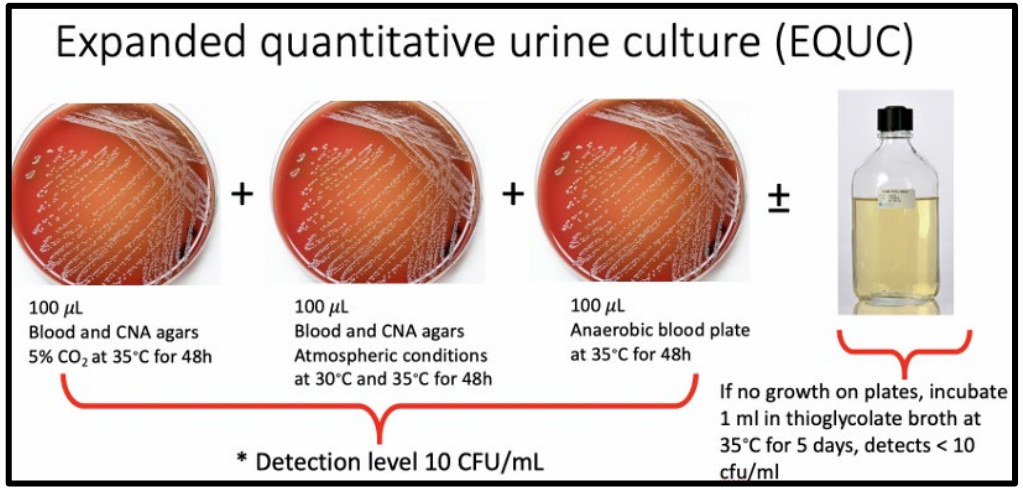
First developed in 2014

Know Your Culture

Standard Urine Culture (SUC)

- 1 µl plated
- ~2 types growth media
- 18- 24 hour incubation
- 1 atmospheric condition

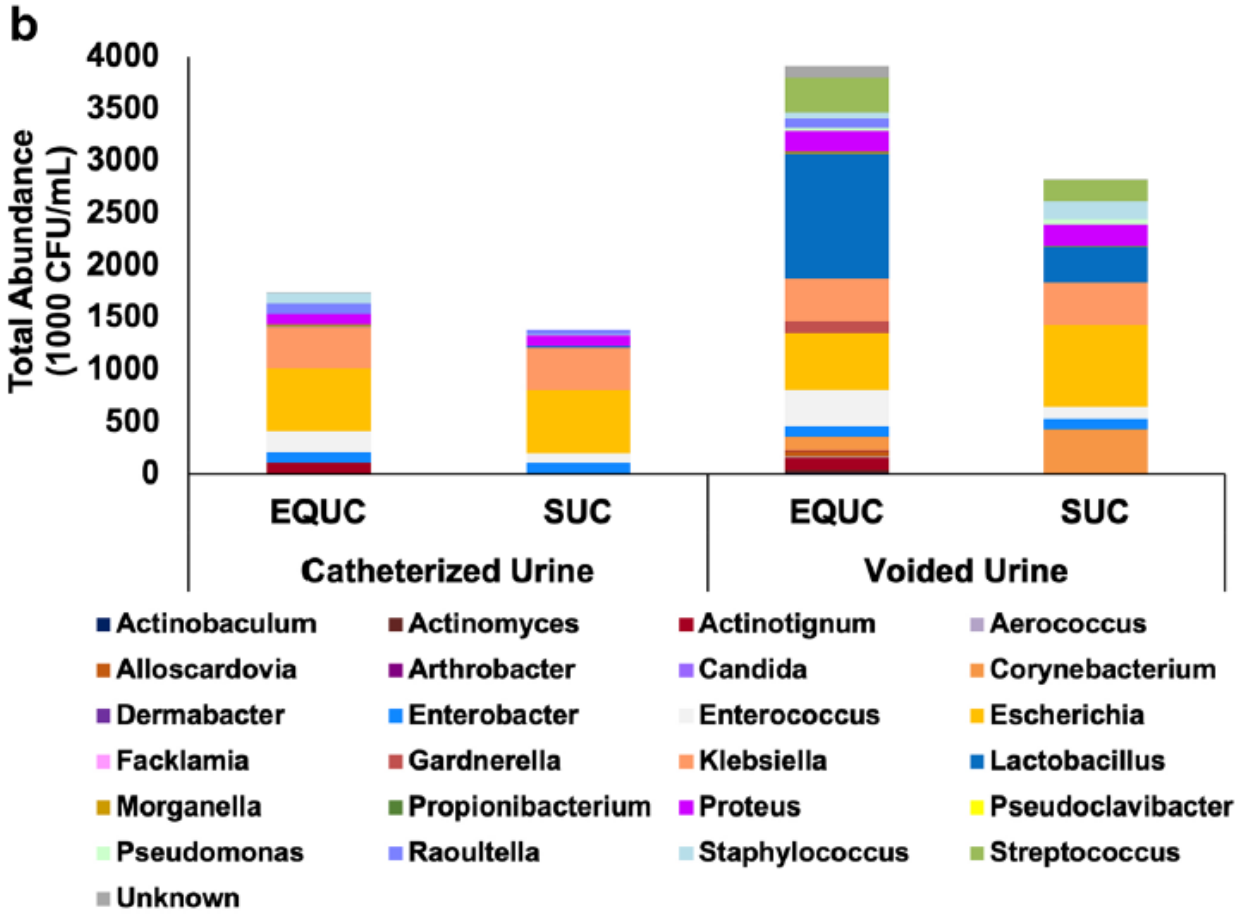




Expanded Quantitative Urine Culture (EQUC)

- 10- 100 µl plated
- 4+ types of growth media
- Up to 48 hour incubation
- Variety of atmospheric conditions

A Quick Look at SUC vs EQUC



More, and additional, bacteria identified with EQUC than SUC

Workflow 1: UTI Culture (2 -3+ days)

Plate

- Whole sample (ex: urine)
- Known quantity

Incubate

- Read plates for growth
- Number of organisms matters

Isolate

- Separate organisms if more than one present

ID/AST

- Preliminary ID
- Susceptibility testing (automated or manual)

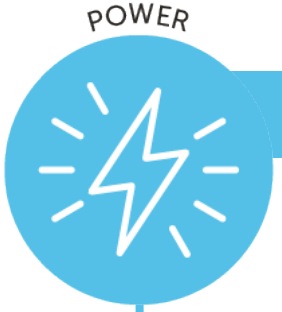
Analysis

- Ensure results “make sense”

Day 1

Day 2 +

UTI Culture: Power & Wisdom



Power

"See" bacteria/yeast/fungi

- Different organisms look different
- Can tell if 1 or more types growing

Standardized; decades of experience/data

- Since 1950s

Well understood

- Well… since 1950s!

Some automation available

- Helps to speed up process
- Standardizes result determination

"Cheap"

Wisdom

Miss pathogens/not sensitive

- "E. coli and friends"

Turn around time

- 2-3+ days

Traditionally call specimens with more than 1-2 organisms as "mixed flora" or "contaminated"

- Can be 1/3 of samples submitted or higher

Phenotypic Resistance Testing (Culture; Automated Methods): Power & Wisdom

POWER

Power

"See" S/I/R (susceptible, intermediate, resistant)

Directly testing the organism causing disease

Decades of data correlating phenotype to treatment/patient outcomes

"Easy to use" clinically

- This is what clinicians are used to reacting to

WISDOM

Wisdom

Takes time

- organism must grow

Need a decent amount of organism

- Not usually an issue with UTIs

Does not exactly predict how the organism/drug combination will behave in the body

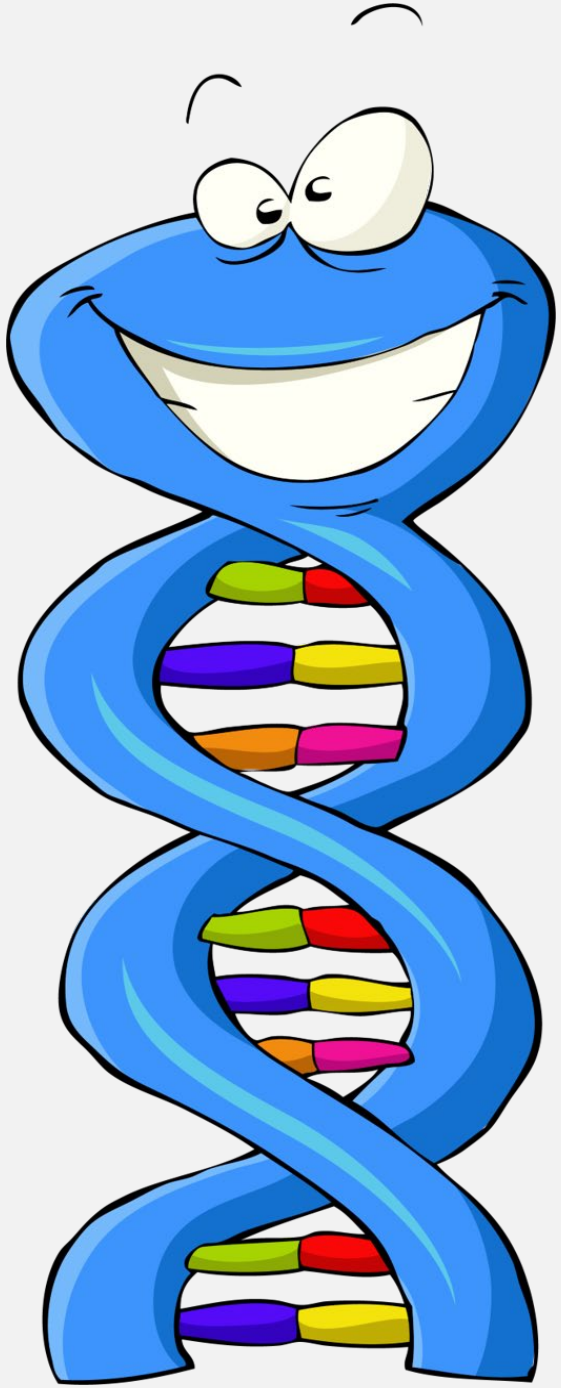
- It is the best we have

"Reading variability"

- Some methods not automated; technician reads results

Does not reveal mechanism of resistance

Traditionally looks at organisms independently



Method 2: PCR (Genotypic)

"Molecular photocopying"

- Amplify/copy small segments of microbial DNA
- Short DNA sequences are used to "interrogate" the sample
  - Is that same sequence in the sample??
  - This method can be used for organism or resistance gene detection
- If that sequence is found, it is amplified/copied over and over again
  - Light is released so that the sample can be analyzed and targets detected
- If that sequence is not found, nothing happens
  - No light is released

Workflow 2: PCR (~1 day)

Sample

- Whole sample (ex: urine)
- Isolated organism

Extract & Purify DNA

- Concentrate
- Remove interferants

Amplify DNA

- "Look" for specific microbial DNA sequences
- Make millions of copies

Detection

- Occurs in "real time"
- Designed so that light is released as specific DNA is found

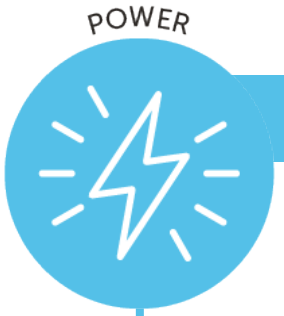
Analysis

- Do results make sense?

Day 1

Note: Not all PCR methods use extraction

PCR: Power & Wisdom



Power

Sensitive

- Detects everything regardless of number of organisms present (think polymicrobial specimen)

Fast

- Some PCRs can run in less than 20 minutes (not yet for UTI… sorry!)

Detect multiple organisms

- Does not matter how fast they grow/if they interfere with each other

Wisdom

“Too sensitive”

- Especially if clinically relevant cutoffs are not set
- Can detect down to very little organism

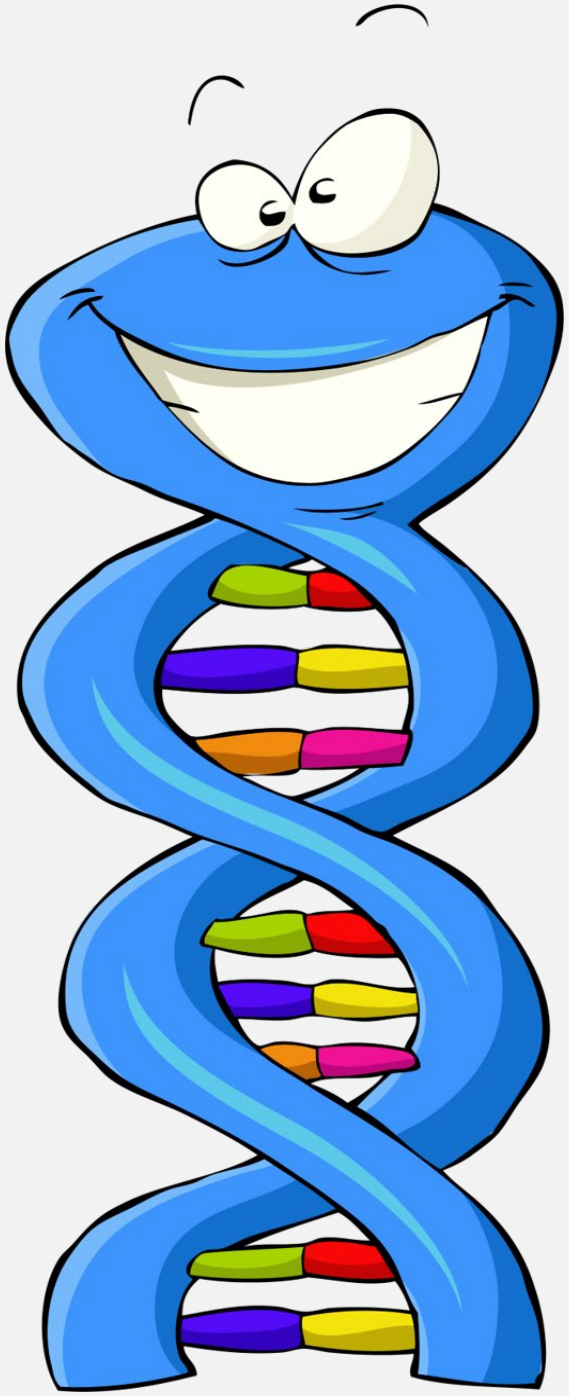
Only detect what you’re looking for

- Will not detect yeast if yeast is not in the PCR

Complex workflow

- Small amounts of liquid
- Risk of contamination

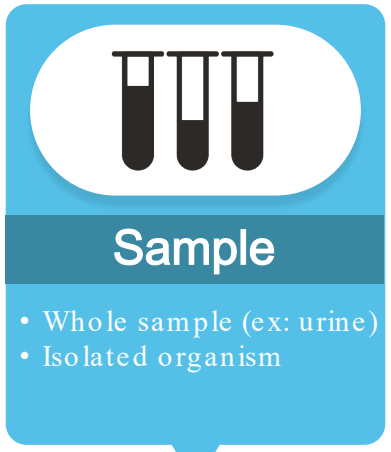
Moderately expensive



Method 3: Next Generation Sequencing (Genotypic)

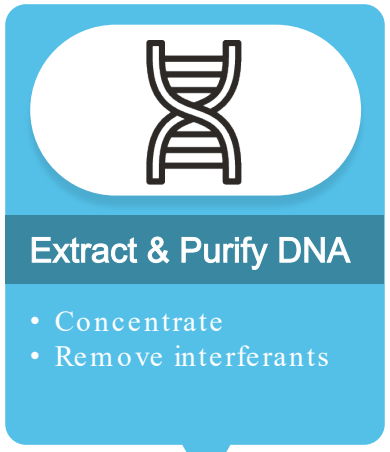
- “Reading” the DNA present in a sample
- Looking for specific sequences and/or small changes in DNA
- Can be used to determine what organism(s) are present in the sample and if resistance genes are present

Workflow 3: Next Generation Sequencing (3+ days)



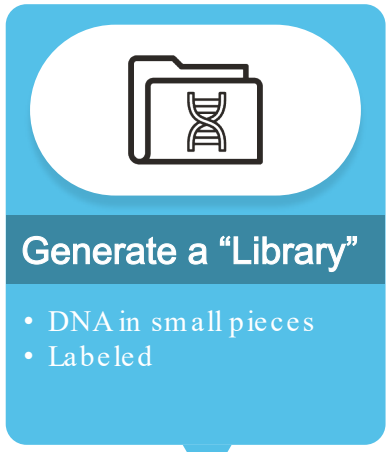
Sample

- Whole sample (ex: urine)
- Isolated organism



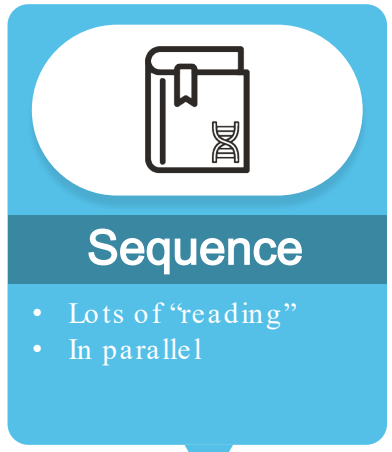
Extract & Purify DNA

- Concentrate
- Remove interferants



Generate a "Library"

- DNA in small pieces
- Labeled



Sequence

- Lots of "reading"
- In parallel

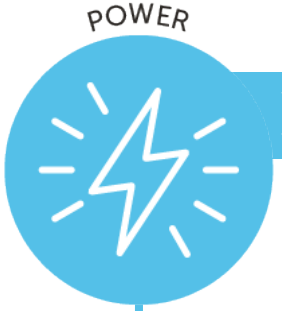
Analysis

- Reassemble "reads"
- Interpret data*

Day 1-2 — Day 2 — Day 3

* "Interpret data" does not convey the amount of effort that people and software use to analyze the data

Next Generation Sequencing: Power & Wisdom



Power

Sensitive

- Detects everything regardless of number of organisms present (think polymicrobial specimen)

“See” entire resistome (entire genome that accounts for antibiotic resistance)

Provides a wealth of information

Wisdom

Detect everything

- May or may not be clinically relevant

Slow (for now…)

“Too sensitive”

- Especially if cutoffs are not set
- Can detect down to very little organism

Expensive

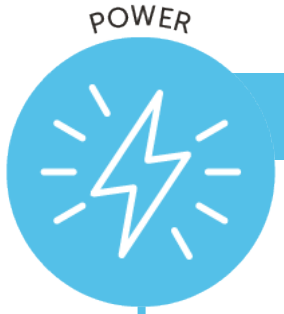
Interpretation can be complex

Results can be database/pipeline dependent

Lack of expertise in Clinical Microbiology laboratory

Complicated workflow

Genotypic Resistance Testing (PCR; Next Generation Sequencing): Power



Power

Quick

- PCR
- Sequencing can take more time but it's getting faster

Low amount of organism needed

Help illicit an unknown resistance mechanism (aid in treatment)

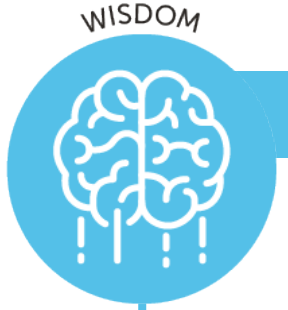
- Knowledge becomes useful for others and "next time"

Knowing mechanism of resistance can help better refine treatment

Lots of investment/research in these technologies

- Everyone wants an answer faster!

Genotypic Resistance Testing (PCR; Next Generation Sequencing): Wisdom



Wisdom

PCR only detects what you're looking for

Sequencing produces a lot of data to analyze/interpret

- Need to link resistance genes to phenotypes
- There is a lot we do not know yet (but we are learning)

Cannot fully predict S/R (no I); accuracy varies widely

- If not detected, "S" is presumed, even though resistance happens through other mechanisms
- If detected then assume "R" (unknown if it would be expressed/turned on)
- Just because you found it does not mean it is "turned on" (expressed)
- No "I" category ("I" can be useful information in complicated treatment cases)

How to separate out what marker belongs to what organism when testing an entire sample (with some technologies)

Not as standardized

Lacking comprehensive outcome studies

Complex interpretations

Results can be database/pipeline dependent

Unknown how to predict resistome interaction in polymicrobial infection

Resistance based on a lot of factors: organism, gene expression, interplay of multiple mechanisms, antimicrobial agent

Assumptions currently made, such as only one gene responsible for resistance

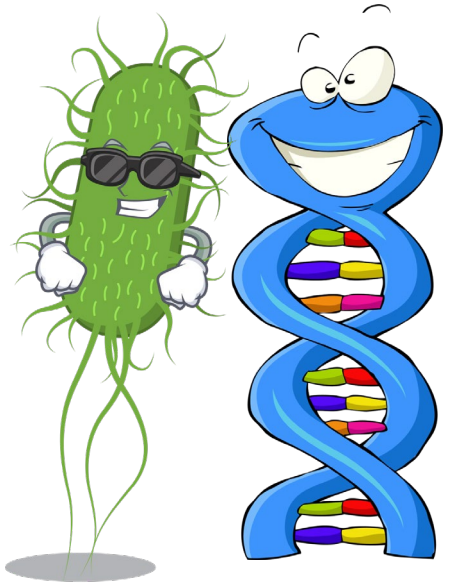
Mixed Methods: Increasing Power, Using Wisdom

PCR for organism detection (and maybe some resistance markers);culture for susceptibility testing

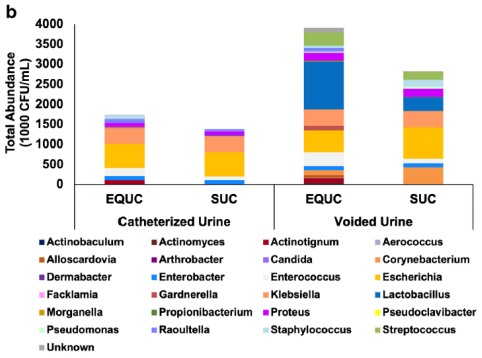
- Speed of PCR (turn around time)
- Phenotypic susceptibility from culture

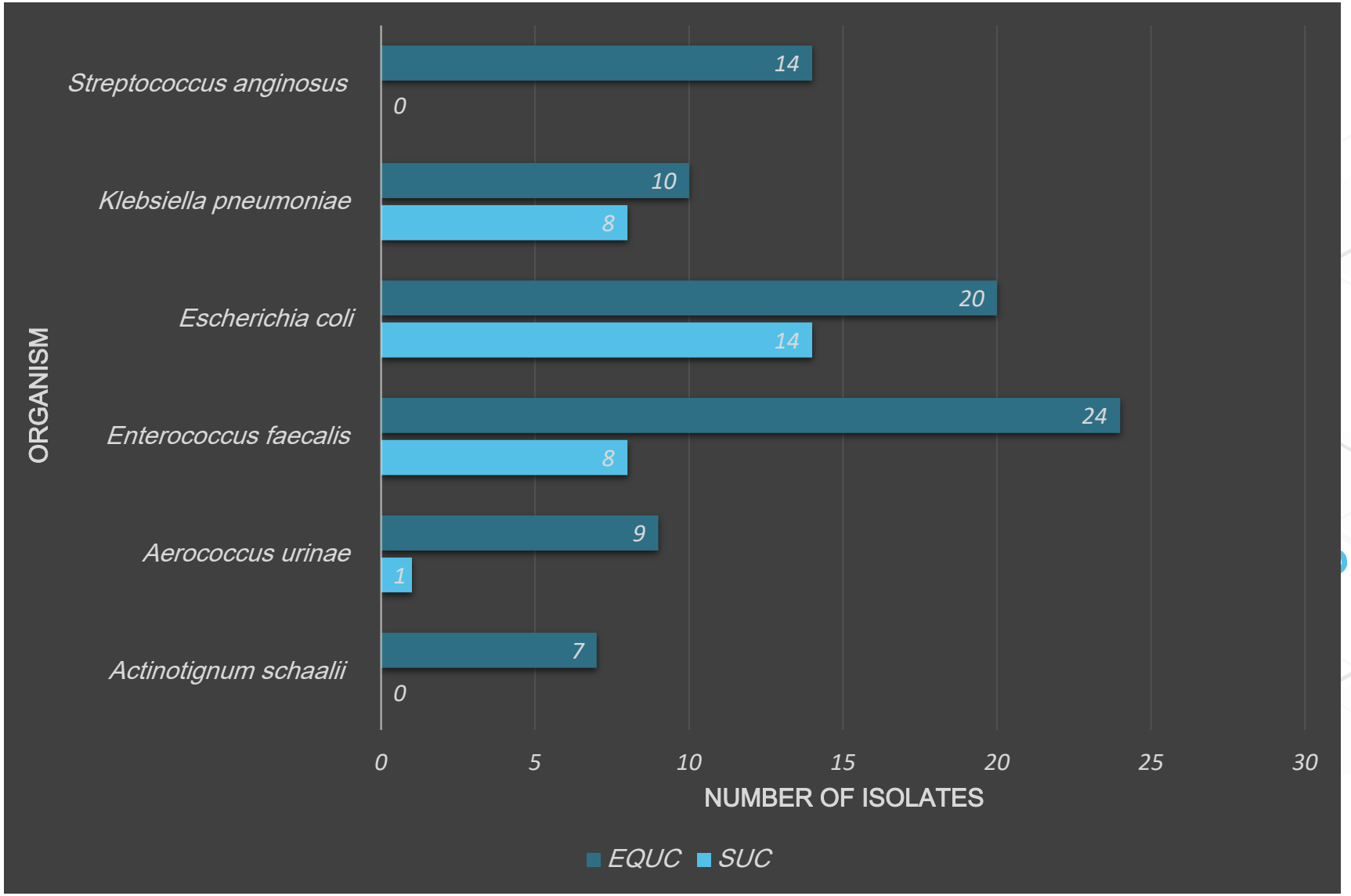
PCR for organism detection (and maybe some resistance markers); then sequencing for the “resistome” (and maybe expanded organism detection)

- Speed of PCR (turn around time)
- Depth of information from sequencing

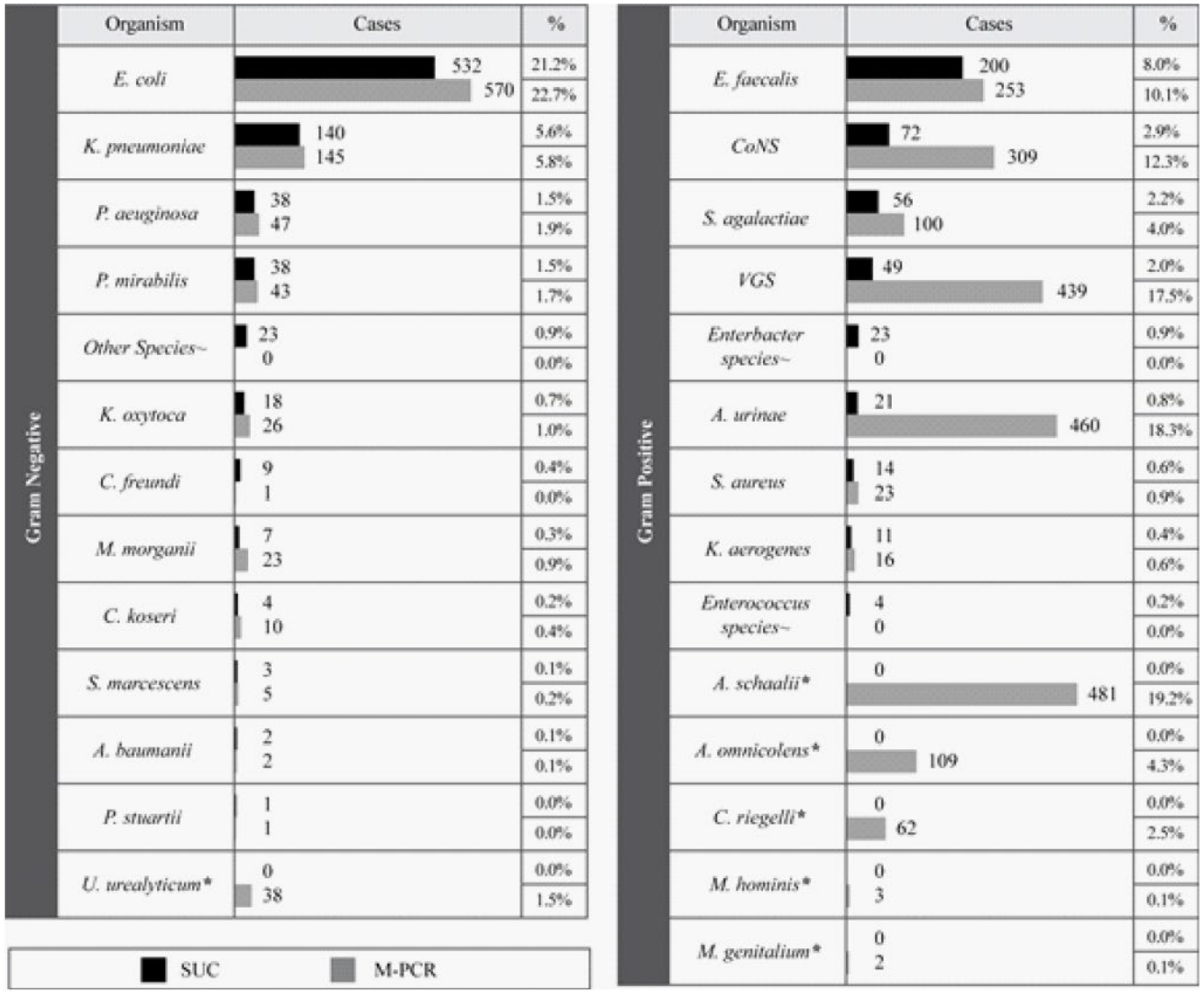


“Hidden” Bacteria - SUC vs EQUC



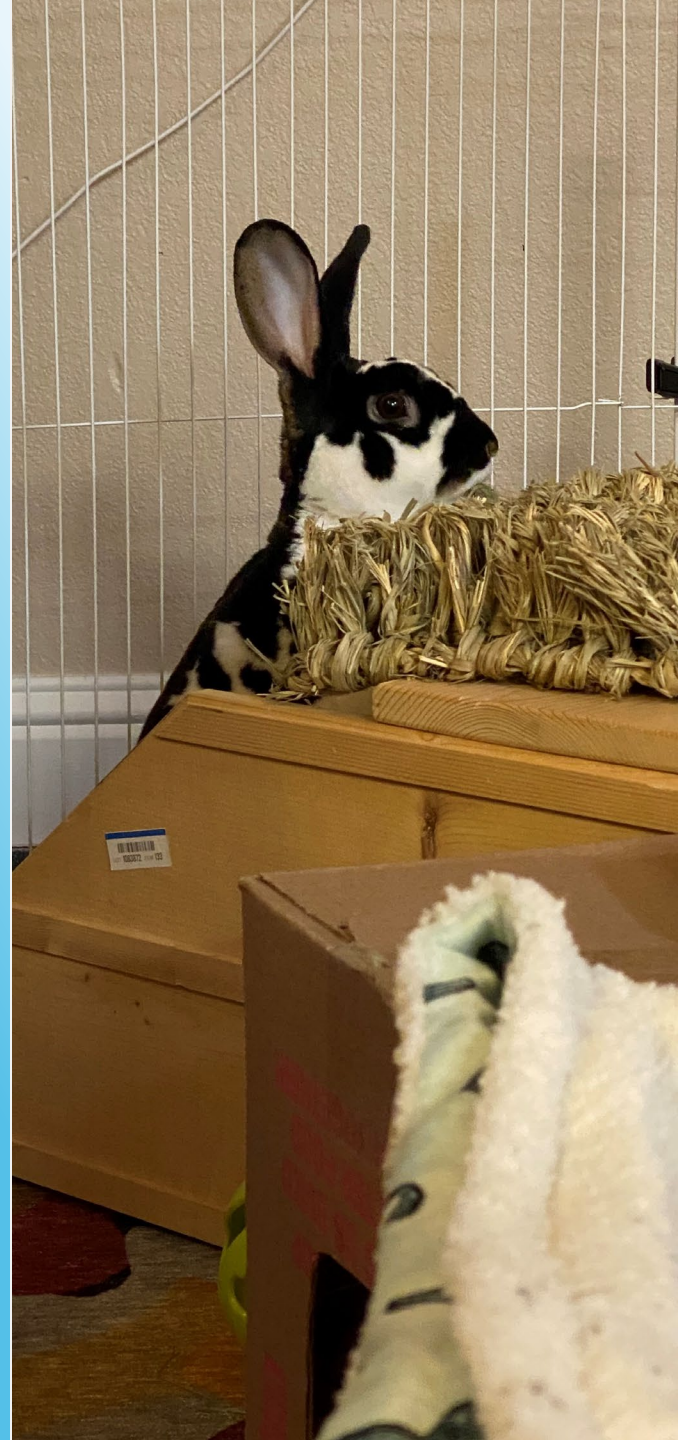


“Hidden” Bacteria – SUC vs PCR



	Organism	Cases	%
Gram Negative	*E. coli*	532 / 570	21.2% / 22.7%
	K. pneumoniae	140 / 145	5.6% / 5.8%
	P. aeuginosa	38 / 47	1.5% / 1.9%
	P. mirabilis	38 / 43	1.5% / 1.7%
	Other Species~	23 / 0	0.9% / 0.0%
	K. oxytoca	18 / 26	0.7% / 1.0%
	C. freundi	9 / 1	0.4% / 0.0%
	M. morganii	7 / 23	0.3% / 0.9%
	C. koseri	4 / 10	0.2% / 0.4%
	S. marcescens	3 / 5	0.1% / 0.2%
	A. baumanii	2 / 2	0.1% / 0.1%
	P. stuartii	1 / 1	0.0% / 0.0%
	*U. urealyticum**	0 / 38	0.0% / 1.5%
Gram Positive	*E. faecalis*	200 / 253	8.0% / 10.1%
	CoNS	72 / 309	2.9% / 12.3%
	S. agalactiae	56 / 100	2.2% / 4.0%
	VGS	49 / 439	2.0% / 17.5%
	Enterbacter species~	23 / 0	0.9% / 0.0%
	A. urinae	21 / 460	0.8% / 18.3%
	S. aureus	14 / 23	0.6% / 0.9%
	K. aerogenes	11 / 16	0.4% / 0.6%
	Enterococcus species~	4 / 0	0.2% / 0.0%
	*A. schaalii**	0 / 481	0.0% / 19.2%
	*A. omnicolens**	0 / 109	0.0% / 4.3%
	*C. riegelli**	0 / 62	0.0% / 2.5%
	*M. hominis**	0 / 3	0.0% / 0.1%
	*M. genitalium**	0 / 2	0.0% / 0.1%

SUC M-PCR



“Hidden” Bacteria Examples

Actinotignum (formerly *Actinobaculum*) *schaalii*

- Will not grow under standard culture conditions
- “an underestimated opportunistic co -pathogen that probably causes UTIs and urosepsis, particularly in elderly patients or patients predisposed for UTIs.”

Enterococcus faecalis

- Slower growing, pinpoint colonies; can easily be “hidden” in standard culture conditions by other bacteria
- Commonly seen with other bacteria
- Research suggests presence can cause *E. coli* to be more pathogenic

Other Gram - positive bacteria

- Harder to grow under standard culture conditions

The Big Summation: Overall Method Comparison




	Standard Urine Culture (SUC)	Expanded Quantitative Urine Culture (EQUC)	Polymerase Chain Reaction (PCR)	Next Generation Sequencing (NGS)
Type of Diagnostic Tool	Phenotypic; developed in 1880s	Phenotypic (advanced); developed in 2014	Genotypic; developed in 1970s	Genotypic (advanced); developed in 2004
Methodology	Grow bacteria and fungi on agar plates with one standard growth condition	Grow atypical bacteria and fungi on agar plates with modified growth conditions	Use short DNA sequences to look for specific bacterial and fungal sequences	"Reads" DNA and matches sequences to bacteria and fungi in curated databases
Detection Highlights	Good at detecting predominant UTI pathogens; not good with other UTI pathogens or polymicrobial infections	Good at detecting pathogens not detected with SUC and polymicrobial infections; may also detect non-pathogens more easily	Good at detecting polymicrobial infections; only detects what the test is looking for	Good at detecting all pathogens in a sample and lists by dominance; detects non-pathogens
Antibiotic Sensitivity Highlights	Phenotypic; bacteria that causes infections used to identify which antibiotics should clear the infection	Phenotypic; bacteria that causes infections used to identify which antibiotics should clear the infection	Genotypic; only detects resistance genes that are on the panel; presence/absence of a gene does not always predict susceptibility	Genotypic; identifies the "resistome" (all known resistance genes present in the sample); presence/absence of a gene does not always predict susceptibility
Specimen Management	Sensitive to time and temperature (organisms need to grow)	Sensitive to time and temperature (organisms need to grow)	Not as sensitive to time and temperature (looking for DNA; organism may or may not be alive)	Not as sensitive to time and temperature (looking for DNA; organism may or may not be alive)
Turn Around Time (from the time the laboratory receives the urine)	2+ days (longer for more complicated organisms/workup)	2+ days (longer for more complicated organisms/workup)	1 day	3-5 days
Cost	$-$$	$$	$-$$	$$$$

A Journey Through the World of UTIs

Secret Life of P:
A quiz about UTIs

UTI Basics:
What you need to know about UTIs

New and Exciting:
What's new in the world of UTIs?

Into the Unknown:
Making sense of all the testing options

Two Roads:
A Patient Case Study

Finish!

Case Studies

Using your mad lab skills

Case Study: Instructions

1. Gather with 3 or 4 neighbors.
2. You'll receive a scenario card: either a culture scenario OR a PCR scenario.
3. Read through the scenario card. (Assume that the patient, Gloria, is symptomatic and meets all criteria for testing.)
4. Read and discuss the questions on the card.
5. Be prepared to share your thoughts!

Case Study Debrief – Overall Points

There is no "wrong" answer… every case is different!

Treat, Because…

- Could get worse
- Avoid admitting patient to hospital
- Worried about Urosepsis
- With an antibiotic that has worked in the past

Wait to Treat, Because…

- Gloria has been on so many antibiotics
- Will antibiotics continue to work?
- Maybe no change in condition while waiting

Treat vs wait big message… it's an UNKNOWN!!!

Hard to know what is going on with some patients

- Can't always communicate

Which is Worse…?

- Treat empirically and find out you're wrong?
- Not treat and get worse?

Impact

- She starts to feel better
- Develop more resistance
- Wipe out normal flora, *C. difficile* now becomes a problem

Stewardship at Your Institution

- Do you have a plan? How does treating/not treating Gloria affect the plan?
- Is it a "box to check"?

Case Study Debrief – Culture vs. PCR

Culture

Culture expectation

- Turn around time… how long?
- Do you expect a “usable” result? How often?
- Know which antibiotics to use to treat infection

Culture bonus points:

- Resend sample/start all over
- Decide to treat if you have not already
- Continue empiric treatment if you started

PCR

PCR expectation

- Fast/faster turn around time
  - Wait to treat?
- More reliable results?
  - Does a “sense of predictability” help?
  - No “contamination/mixed flora”
- Do you expect susceptibilities… a treatment “answer”?
  - Is saying “don’t use this antibiotic” helpful? What about “use this antibiotic”?
- More accurate results?
- Is every PCR (or test) the same?
- Expect a positive result
  - “Know” it’s a UTI and then the result is negative… then what??

PCR bonus points:

- What is *A. schaalii* again?!? What does it mean if culture doesn’t detect this?
- What does it mean to detect resistance genes?
- If you feel that you can’t use vancomycin or the beta - lactam antibiotics (ex: penicillins, cephalosporins), what do you do?

Case Study Debrief – Double Bonus

Factors to consider:

- Turn around time
- Accuracy
- Reliability
- Predictability of when results are available
- Antibiotic results

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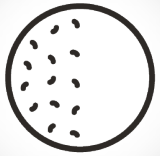

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The Final Burn

Q&A

Burning Questions?

What questions do you have?

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Finish!

abambach@cirrusdx.com

Thank you!

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Polymicrobial Interactions

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