YOUR FRIENDS IN THE LAB:
HOW MICROBIOLOGY CAN HELP
IN THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE

Dr. Jessica Minion, Medical Microbiologist
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OBJECTIVES

• Beyond Screening: what is the role of microbiology in the fight against antimicrobial resistance?

• Antibiograms & Resistance

• Interventions based in the Lab
DECLARATION OF CONFLICTS OF INTEREST

- none
PAN-CANADIAN FRAMEWORK ON AMR
## PAN-CANADIAN FRAMEWORK ON AMR

<table>
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<tr>
<th>MICROBIOLOGY</th>
<th>Surveillance</th>
<th>Stewardship</th>
<th>Infection Prevention &amp; Control</th>
<th>Innovation</th>
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<td>POCT</td>
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<td>Defining Resistance</td>
<td>Selective Reporting</td>
<td>Outbreaks</td>
<td>CRISPR</td>
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<td>New Diagnostics</td>
<td>Transmission</td>
<td>Metagenomics</td>
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KEY FEATURES OF ANTIBIOGRAMS

- Cumulative Susceptibility Report, for a given population over a specific period of time.
- Report of prior laboratory results, often used to predict future results
- Used for:
  - Empiric treatment decisions
  - Monitoring trends in resistance
  - Targeting antimicrobial stewardship initiatives & monitoring the effectiveness of interventions whose goal is to reduce antimicrobial resistance
  - Analysis of subgroups to determine drivers of resistance in your community
### WHAT GOES INTO AN ANTIBIOGRAM?

<table>
<thead>
<tr>
<th>Description</th>
<th>Requirement</th>
</tr>
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<tbody>
<tr>
<td>Last Word</td>
<td>Include only final, verified test results</td>
</tr>
<tr>
<td>Trustworthy</td>
<td>Include only species with testing data for at least 30 isolates overall</td>
</tr>
<tr>
<td>Relevance</td>
<td>Include only diagnostic (not surveillance) isolates</td>
</tr>
<tr>
<td>Duplicity</td>
<td>Eliminate duplicates by including only the first isolate of a species per patient per analysis period irrespective of body site or antimicrobial profile</td>
</tr>
<tr>
<td>Include</td>
<td>Include only antimicrobial agents routinely tested, not supplemental agents selectively tested on resistant isolates only</td>
</tr>
<tr>
<td>Combine</td>
<td>Reports %S; Intermediate and Resistant interpretations are combined</td>
</tr>
</tbody>
</table>
WHAT CAN INFLUENCE YOUR ANTIBIOGRAM RESULTS?

- Antibiograms will be affected by:
  - Patient population served
  - Lab utilization patterns
  - Lab protocols and policies
  - Temporal outbreaks
PRACTICAL EXAMPLES
MS. D

- 70 yr old woman, living in long term care in Regina
- Diagnosed with lower UTI, urine specimen sent to lab
- Started on Ciprofloxacin

- Next day, lab reports culture + $10^8$ E.coli
- Susceptibility to follow

- Treatment OK?
### E. coli cumulative susceptibility to Cipro in RQHR:
- Overall: 82% (n=5024)
- Urine Specimens: 83% (n=4787)
- LTC residents: 55% (n=350)

### Other options?
- Nitrofurantoin
  - Overall: 97%
  - Urine Specimens: 96%
  - LTC residents: 93%
• 48 yr old man on chemotherapy for ALL
• Diagnosed with sepsis, blood cultures sent to lab
• Started on PipTazo

• Next day lab reports culture + GPC clusters, 2 hours later presumptive ID = *Staphylococcus epidermidis*
• Susceptibility to follow

• Treatment OK?
Other options?

- Vancomycin 100%

But PipTazo isn’t on the Antibiogram!

### Staphylococcus epidermidis cumulative susceptibility to beta-lactams in RQHR:

- Overall: 30% (n=153)
- Blood: 25% (n=45)
BABY G

• 3 month old female
• Diagnosed with pneumonia, respiratory specimen sent to lab
• Started on azithromycin

• Lab reports Gram:
  • 4+ Polymorphonuclear cells
  • 2+ Squamous epithelial cells
  • 4+ Gram positive diplococci
  • 1+ mixed morphotypes

• Treatment OK? Need to change?

But Azithromycin isn’t on the Antibiogram!?

What is the likely organism?
### Regina Qu’Appelle Health Region ANTIBIOGRAM

**All Patients**

**January 1, 2016 - December 31, 2016**

#### All Specimens — % Susceptible

<table>
<thead>
<tr>
<th>Gram-Positive Bacteria</th>
<th>Penicillin PO</th>
<th>Penicillin IV NM</th>
<th>Penicillin IV M</th>
<th>Ceftriaxone IV NS</th>
<th>Ceftriaxone IV M</th>
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</thead>
<tbody>
<tr>
<td>Staphylococcus aureus, all</td>
<td>681</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>96</td>
</tr>
<tr>
<td>- methicillin-susceptible</td>
<td>1379</td>
<td>84</td>
<td>86</td>
<td>88</td>
<td>91</td>
</tr>
<tr>
<td>- methicillin-resistant (MRSA)</td>
<td>546</td>
<td>R</td>
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<tr>
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<td>153</td>
<td>61</td>
<td>70</td>
<td>78</td>
<td>86</td>
</tr>
<tr>
<td>Viridans group streptococci</td>
<td>33</td>
<td>91</td>
<td>97</td>
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</tr>
<tr>
<td>Enterococcus species (urine)</td>
<td>555</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>160</td>
<td>70</td>
<td>81</td>
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</tr>
<tr>
<td>Enterococcus faecium</td>
<td>141</td>
<td>70</td>
<td>75</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>145</td>
<td>83</td>
<td>91</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
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<tr>
<td>Group A Streptococcus</td>
<td>48</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>Streptococcus anginosus group</td>
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#### Regina Qu’Appelle Health Region ANTIBIOGRAM

**Pediatrics (≤17 years)**

**January 1, 2014 - December 31, 2016**

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**Other options?**

- Septra 85-90%
- Beta-lactams

**Streptococcus pneumoniae** cumulative susceptibility to macrolides in RQHR:

- Overall: 75% (n=162)
- Pediatrics: 70% (n=114)
Can you walk in your friend’s shoes?

You *CAN* … but you shouldn’t if you don’t have to

Variability in microbial populations can be significant in different geographic locations

Just like any infectious disease!

Added variability in ordering practices, transmission dynamics, lab protocols

Consider patient characteristics/demographics
INFORMATION AT THE POINT OF CARE

• Antibiograms app – available FREE for iPhone and Android devices

• Search app store for “Antibiograms” and download onto mobile device

• Open database file (.db) from email/website
  • ‘What program do you want to open this with?’

• Requires your lab to create a .db file
  • LABS! Call me if you need help creating your database file
### Escherichia coli

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<td>Amoxicillin-Clavulanic acid</td>
<td>97% susceptible</td>
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<tr>
<td>Ampicillin/Amoxicillin</td>
<td>98% susceptible</td>
</tr>
<tr>
<td>Cefazolin</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Cephalaxin</td>
<td>66% susceptible</td>
</tr>
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<td>Ciprofloxacin</td>
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</tr>
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<td>Ertapenem</td>
<td>100% susceptible</td>
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<td>97% susceptible</td>
</tr>
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<td>78% susceptible</td>
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#### Footnote

2.2% of E. coli (n=129) were +ESBL (extended spectrum beta-lactamase)

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### Enterobacter cloacae

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#### Footnote

This organism may produce an inducible beta-lactamase. Treatment with a penicillin or cephalosporin can result in clinical failure despite in vitro susceptibility.
**SUBGROUPS**

- **Patient Type Subgroups**
  - Inpatient
  - Outpatient
  - LTC
  - Emergency
  - ICU
  - Pediatric, Adult, Senior
  - Length of Stay?

- **Specimen Type Subgroups**
  - Blood
  - Urine
  - Excluding Urine

- **Specialty Organisms**
  - Anaerobes
  - Yeast
CONTINGENT ANTIBIOGRAMS

• The likelihood of at least one antibiotic being susceptible when given in combinations

• E.g. Hypothetical Pseudomonas aeruginosa susceptibility:
  • PipTazo 85%
  • Ceftazidime 80%
  • Cipro 75%

• What 2 drug regimen will yield the highest coverage rates?
  • Given PipTazo=R, what is ceftaz %S?
  • Given Ceftaz=R, what is Cipro %S?
  • etc
WEIGHTED INCIDENCE SYNDROMIC CONTINGENT ANTIBIOGRAM (WISCA)

- Takes into account the site of isolation and provides a weighted susceptibility of all organisms causing a specific infectious syndrome

- E.g. Hypothetical Urinary Tract Infections
  - 80% caused by E.coli
  - 10% caused by Enterococcus
  - 7% caused by other GNB
  - 3% caused by other GPC

Ampicillin Susceptibility
- E.coli 50% S
- Enterococcus 90% S
- Other GNB 85% S
- Other GPC 95% S

Weighted Susceptibility
- 0.8 * 50 = 40
- 0.1 * 90 = 9
- 0.07 * 85 = 5.95
- 0.03 * 95 = 2.85
Sum = 57.8%
DEFINING RESISTANCE
NO LACK OF DEFINITIONS

INTRINSIC vs. ACQUIRED?

PHENOTYPIC vs. GENOTYPIC?

MUTATIONS vs. HORIZONTAL GENE TRANSFER?

MECHANISM OF ACTIVITY?
  - Target modification vs.
  - Antibiotic alteration vs.
  - Restricted target access vs.
  - Global adaptive processes
PHENOTYPIC

• How well does bug grow in presence of drug in vitro
• Yields MIC – “the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation”
• Requires minimum 24 hrs
• Interpretation requires breakpoints
• E.g. microdilution, E-tests, Kirby-Bauer, automated methods

GENOTYPIC

• Detect presence of genes associated with mechanism of resistance
• Needs robust association with phenotype for interpretation
• Rapid
• Interpretation = present/absent
• E.g. PCR, genetic sequencing

DETERMINING SUSCEPTIBILITY
DETERMINING SUSCEPTIBILITY

• What you want to know: “Is my patient’s infection likely to respond to treatment with this antibiotic?”

• What an MIC tells you: “This concentration of antibiotic inhibits visible growth on a plate after 24 hours.”
MIC Interpretations

- Set by organizations with various funding mechanisms
- Can be lengthy, complicated, often controversial processes
- CLSI, FDA, EUCAST

Committees

- Bacterial Metabolism
- Intrinsic Resistance
- Acquired Resistance
- Wild Type
- Growth Characteristics

Pharmacodynamics (Pharmaco-D/K)

- Absorption
- Distribution
- Protein Binding
- Dosage
- Excretion/Metabolism

Patient Information

- Site of Infection
- Immune Status
- Outcome
- Evidence

Microbiology

- Site of Infection
- Immune Status
- Outcome
- Evidence
MULTI-DRUG RESISTANCE / EXTENSIVE DRUG RESISTANCE

Considers resistance to categories of antimicrobials

More patient-oriented

Resistance vs. Non-susceptibility

Phenotypic definitions
# 4 Pillars of Antimicrobial Stewardship

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WHAT’S INSIDE THE BLACK BOX

You collect & send specimen to lab

Based on info we have, gets processed and inoculated onto agar

24hrs+ bacterial growth is detected

Need to get isolated colony to do identification, set up for susceptibility testing

Up to weeks

More info – more help

‘Subs’

“Garbage in, Garbage out

Smear

Earlier results = more impact

CFU – single cell

Earlier results = more impact
DIAGNOSTICS & STEWARDSHIP

BETTER TURNAROUND TIME
- ID & Susceptibility

BETTER DIAGNOSTICS
- Biomarkers of Infection
SPEED IT UP!

- Decreasing TAT in micro lab can result in:
  - decreased antibiotic use
  - decreased inappropriate antibiotic use
  - decreased time to initiating appropriate antibiotic therapy
  - decreased length of stay
  - decreased ICU stay
  - fewer days of antimicrobial therapy,
  - decreased drug costs
  - decreased hospital costs
  - mortality

Rapid Gram stain communication alone decreases mortality due to blood stream infections!

<1 hr (ave 0.1 hr) – 10.1% mortality
>1 hr (ave 3.3 hr) – 19.2% mortality

MANY STUDIES NOW…

Not Comprehensive!
I stopped collecting evidence in 2013…


BETTER TURNAROUND TIME

• Can be achieved through process improvement, staffing changes, laboratory policies

• New testing technology:
  • Peptide Nucleic Acid-Fluoresence In Situ Hybridization (FISH)
  • Real-time Polymerase Chain Reaction Assays (RT-PCR)
  • Matrix-assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF)
  • Broad-based Multiplexed Nucleic Acid Assays for Blood Cultures (Arrays, Panels)

• New laboratory automation
MALDI-TOF

- Decreased time to identification 84.0 vs 55.9 hrs
- Improved time to effective Abx therapy 30.1 vs. 20.4 hrs
- Time to optimal antibiotic therapy 90.3 vs. 47.3 hrs
- Mortality 20.3% vs. 14.5%
- Length of ICU stay 14.9 vs. 8.3 days
- Recurrent bacteremia 5.9% vs. 2.0%

DIRECT PCR

- ID Pharmacist contacted with results
- Time to switch from empiric vancomycin to cefazolin in patients with MSSA 1.7 days shorter
- Length of stay 6.2 days shorter
- Mean hospital costs $21,387 less

TOTAL LAB AUTOMATION

• Robotics can now automate:
  • Culture plate inoculation and streaking
  • Gram smears and staining
  • “Smart Incubators” decrease time to result
  • Image Analysis of culture growth
  • Hands-free discard of negative cultures
  • Digital selection of isolates for work-up
  • Performance of ID and Susceptibility testing

Copan WASP™ Lab:
https://www.youtube.com/watch?v=AFQBPoQZZ9Y
• Benefits often are not realized if implemented by lab alone
  • Synergy when implemented in partnership with Antimicrobial Stewardship Programs, to ensure translation of decreased TAT of results to action

• We need your help!
  • Laboratory interventions and new diagnostics usually COST more money in the lab, while SAVING money outside the lab
  • We can achieve net savings for health system by investing in lab, if done properly.
  • Joint Business Cases which estimate/demonstrate return on investment in clinical area

• WARNING! Buyer beware! Diagnostics market is not well controlled…
  • Do not engage with sales reps who want to bypass your lab personnel
  • Ignore claims of performance made in product inserts
  • I’ve never met a distributor who doesn’t sell “The Best” brand of test X
  • Lab personnel – be skeptical, check references and literature, verify verify verify! RFPs can be your friend.
Antimicrobial Stewardship Strategy: Cascading microbiology susceptibility reporting

The selective suppression of an organism’s susceptibility to broader-spectrum or more expensive secondary agents when it is susceptible to preferred primary agents.

## 4 Pillars of Antimicrobial Stewardship

### Surveillance
- Antibiograms
- Defining Resistance

### Stewardship
- Communication
- Selective Reporting
- New Diagnostics

### Infection Prevention & Control
- Detection
- Outbreaks
- Transmission

### Innovation
- POCT
- CRISPR
- Metagenomics
POINT OF CARE TESTING

Multiplexed point-of-care testing

- Paper
- Array
- Beads
- μFluidic

- Proteins
- Exosomes
- DNA
- Metabolites

Analysis of the multiplexed results

Personalized therapy

Trends in Biotechnology
CRISPR TECHNOLOGY

“Clustered regularly interspaced short palindromic repeats”

- Programmable, sequence-specific genome modification using the RNA-guided nuclease Cas9, delivered by a bacteriophage

- Based on bacterial immune system enzymes – Cas9

  - Target virulence genes?

  - Target antimicrobial resistance genes?

  - Immunize avirulent strains against acquiring resistance genes or virulence factors?
METAGENOMICS

- Looks at all the genetic material in a sample
- More accurately reflects how microbes (and host cells) live and exist together
  - Bugs don’t live in pure cultures, they live in complex communities
  - Genetic diversity exists within strains, yet we deal with clonal cultures
  - Recognizes the large pool of ‘unculturable’ organisms
- Characterization of the Microbiome
  - Which microbiome?
  - Disease associations
  - Cause $\leftrightarrow$ Effect
  - Bug $\leftrightarrow$ Drug
Thank you!!