



Application of New (and some not so new) Molecular Tools

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Introduction

- This talk will focus primarily on the use of typing and diagnostic methods for outbreak investigations.



Molecular Typing

- Has always been, and remains, a key part of outbreak investigations.
- Used to determine if isolates are genetically related.
- It often provides the definitive answer in an investigation.



Molecular Strain Typing Techniques



- **Pulsed-field gel electrophoresis (PFGE)**
- Arbitrarily-primed PCR (rep-PCR)
- Randomly amplified polymorphic DNA (RAPD)
- Multilocus sequence typing (MLST)
- Multilocus variable number tandem repeat analysis (MLVA)
- Plasmid fingerprinting
- DNA sequencing of variable loci

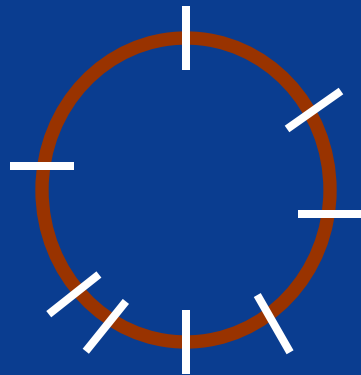


What about resistance patterns?

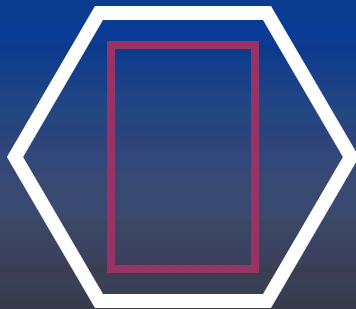
- (Almost) everyone has access to the susceptibility results for the organisms in an outbreak.
- It's helpful, but not at all conclusive.
- Organisms with the same antibiogram may or may not be genetically related because resistance often depends on gene expression and typing generally measures gene presence.

Pulsed-Field Gel Electrophoresis

Each lane = one organism



Bacterial chromosomal DNA
Cleaved with an enzyme



Electrophoresis chamber
Separates DNA fragments



Kb

680

600

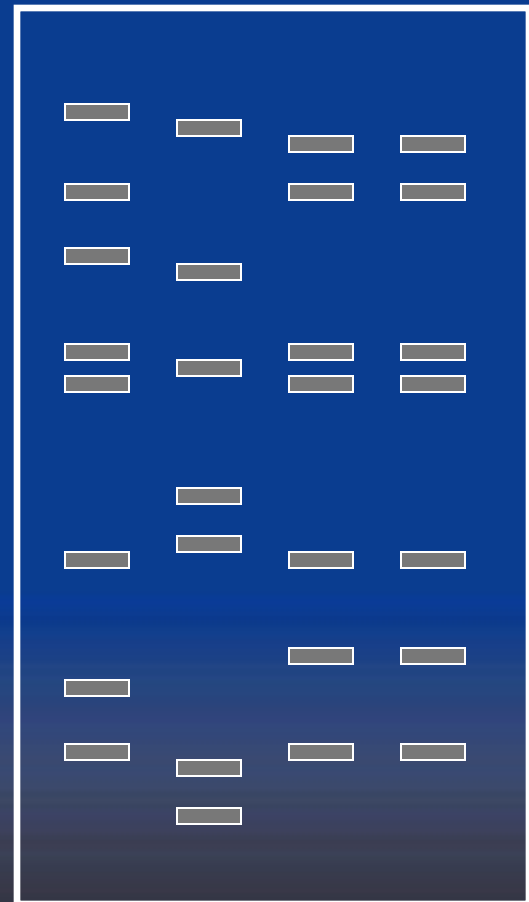
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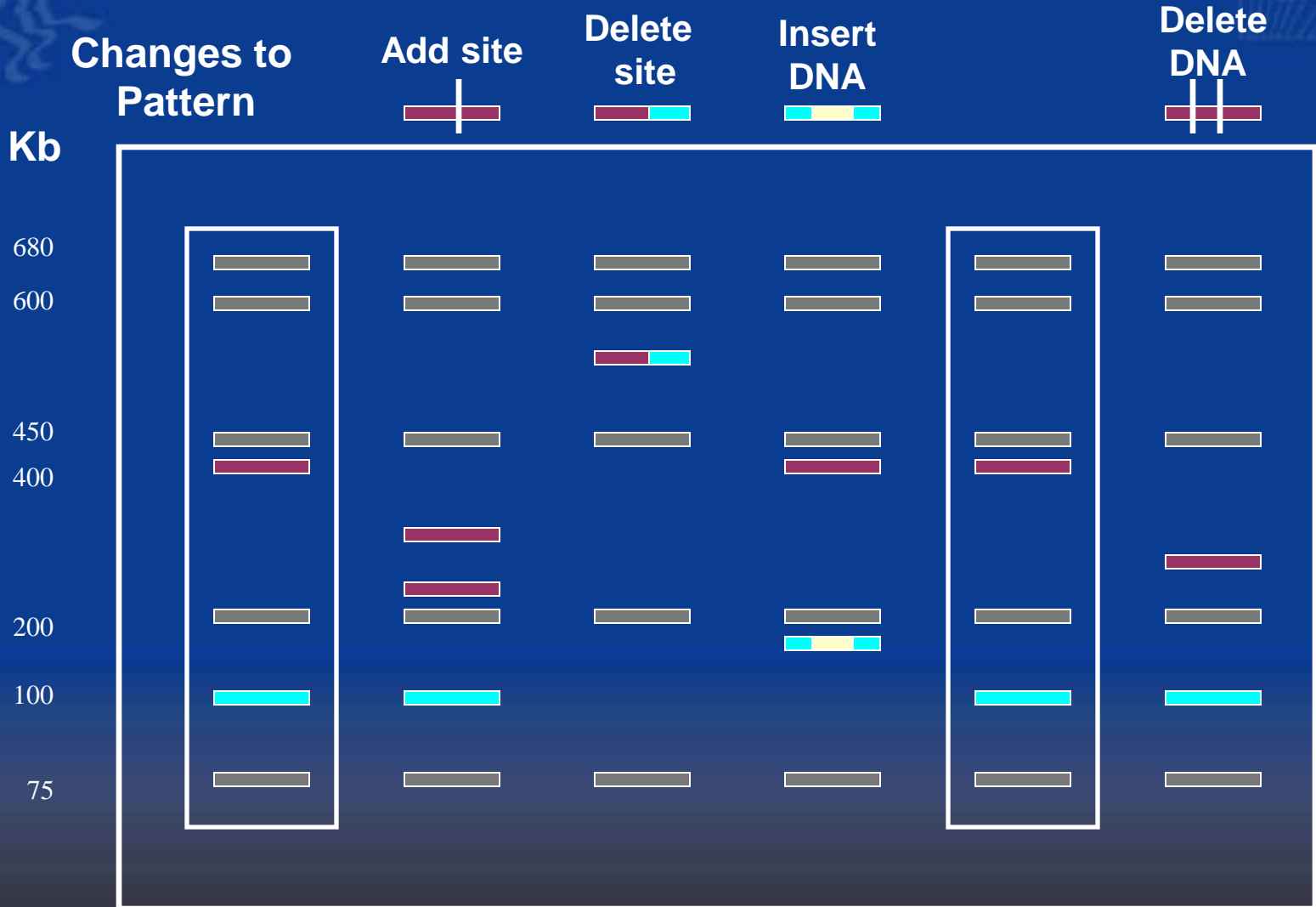
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100

75



Understanding changes in PFGE profiles



Assigning Strain Types-PFGE

Fragment differences	Genetic events	Typing result	Epi correlation
0	0	Indistinguishable (A)	Outbreak related
1-3	1	Closely related (A1)	Probably part
4-6	2	Possibly related (A2)	Possibly part
≥ 7	3	Different (B)	Not

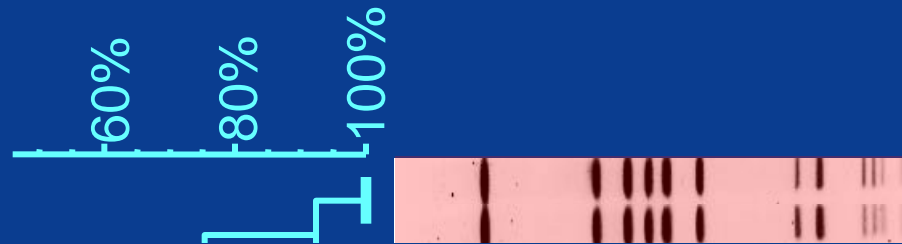


Molecular typing

- Can provide the “slam-dunk” that we all crave in outbreak investigations.
- But, there are challenges:
 - You have to have the organisms
 - It’s expensive and not available everywhere
 - It does not always answer the question!

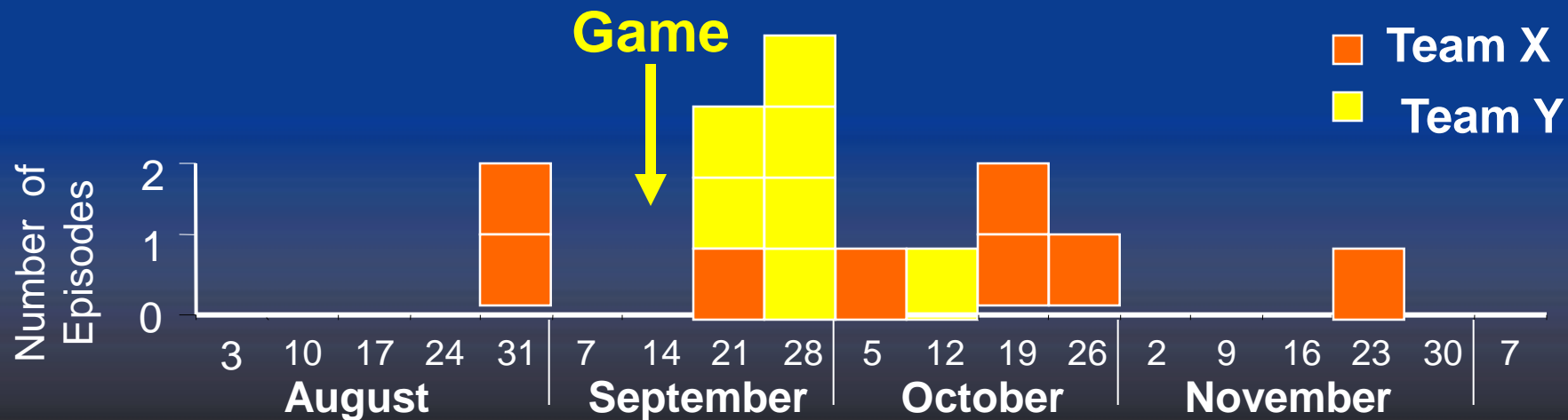
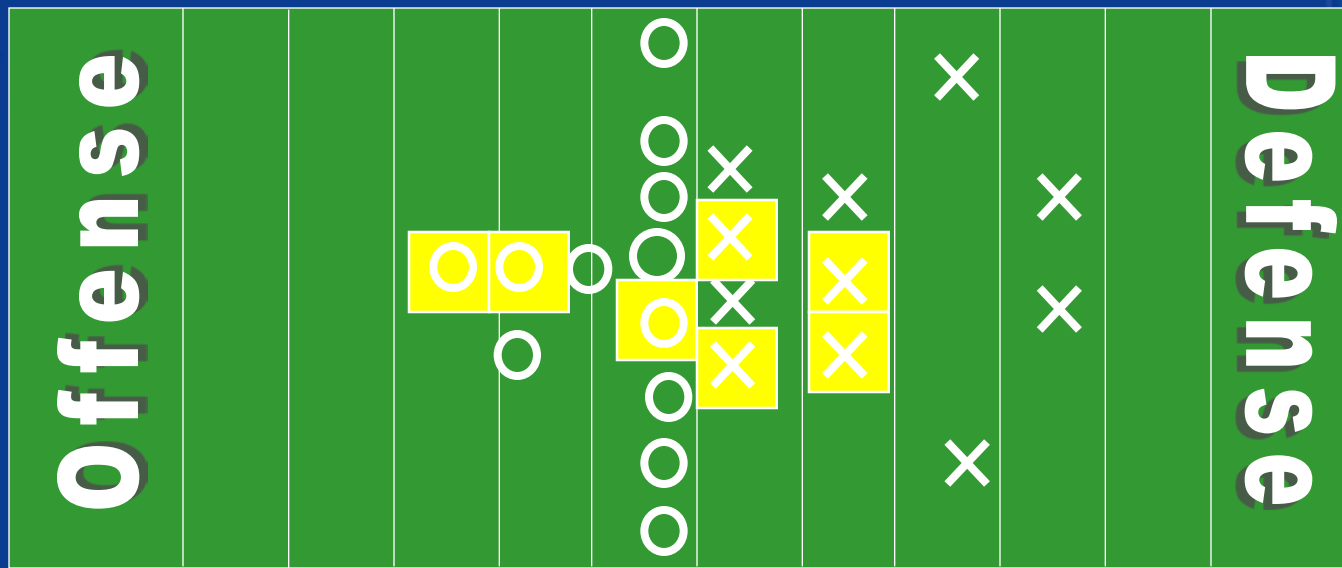
Results – Molecular Epidemiology

Team X MRSA: USA 300



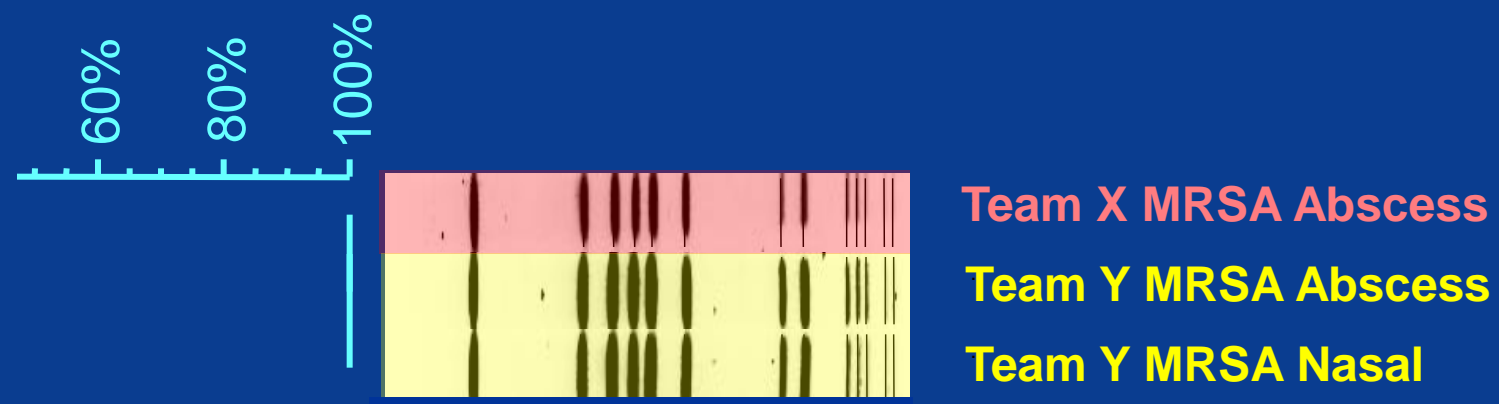
MRSA: Abscess
MRSA: Abscess

Results – Cases (Team Y and Team X)

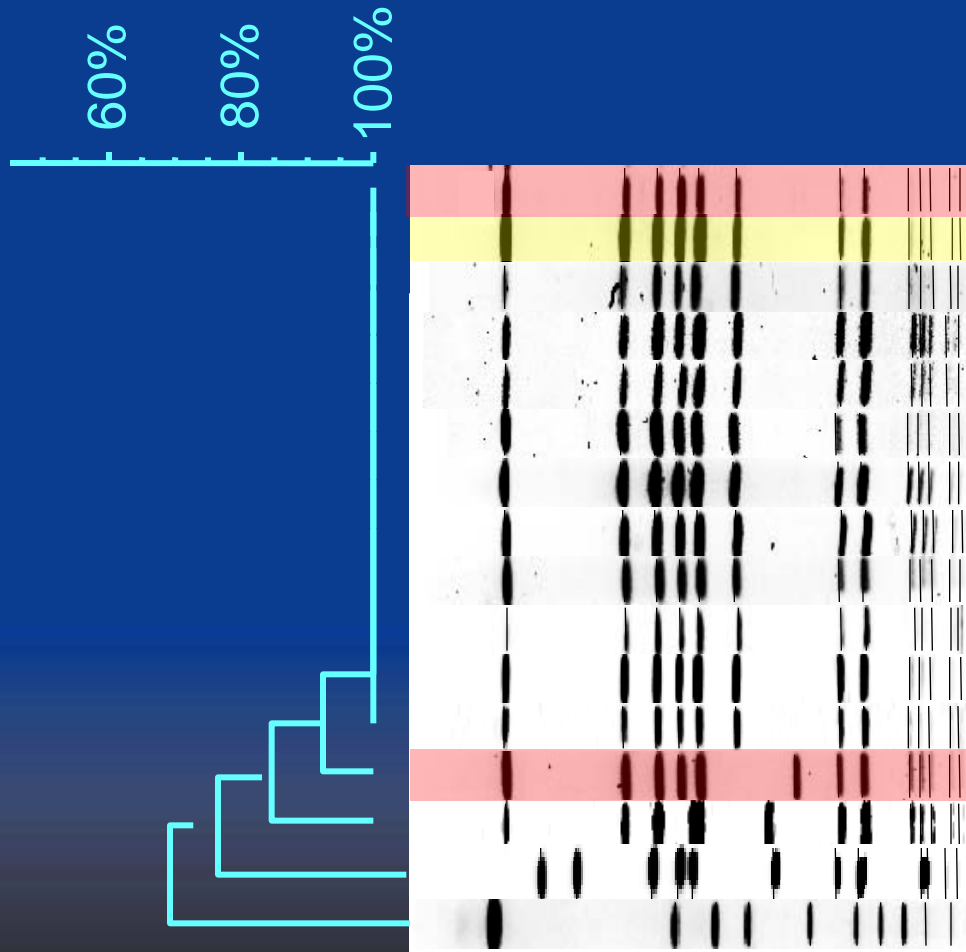




A connection? MRSA from teams X and Y



Yes, but not what we thought!



Team X	MRSA, Abscess
Team Y	MRSA, Abscess
California	College Football
Pennsylvania	HS Football
Colorado	Fencers
California	MSM
Mississippi	Prison
Texas	Jail
Georgia	Prison
Tennessee	Children
Texas	Children
Missouri	Children
Team X	MSSA, Nasal
USA300	
USA100	Hospital strain
USA200	Hospital strain



“Different” may not mean “The end”

- NICU outbreak with three-fold increase in *Pseudomonas* pneumonias.
- Typing showed several different strains.
- We still have a problem!



Molecular typing

- Strain typing data can provide useful data for outbreak investigations
- Typing data is NOT a substitute for a sound epidemiologic investigation
- The two data sets should be used together to provide complementary information



New Diagnostic Methods: Respiratory Infections MRSA

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Outbreaks of Respiratory Viruses

- These occur in the community every year and create infection control challenges in every pediatric setting.
 - Lots of infectious patients
 - Multiple pathogens circulating simultaneously
 - Transmission can occur over distance



Growing List of Pathogens

- Influenza and RSV continue to lead the way.
- But, there is growing evidence that other agents are causing outbreaks as well:
 - Parainfluenza virus
 - Adenovirus
 - Human metapneumovirus
 - Human bocavirus



Diagnostic Challenges

- Diagnosis of respiratory illnesses has been, and remains, challenging.
- Though several methods are available, each comes with pros and cons.



Viral Culture

- Pro:
 - Don't need to know what you're looking for
 - Yields an isolate which can be typed
- Con:
 - Takes time
 - Yield can be low, depending on sample and agent (coronaviruses, rhinovirus)



Direct Fluorescence Antibody (DFA)

- Pro:
 - Quick
 - Relatively easy to perform
 - Good yield in pediatric patients
- Con:
 - Reagents are pathogen specific- you have to know what you're looking for
 - Some sample collection issues



Nucleic Acid Amplification (NAT)

- Pro:
 - Quick
 - Very sensitive
 - Can be automated for high throughput
 - Sample collection less of an issue
- Con:
 - Reagents are pathogen specific
 - Equipment can be expensive



And the Winner Is?

- There is a growing move towards NAT testing for respiratory pathogens.
- The availability of multiplex assays that can detect several viruses at once has further increased enthusiasm.

Multiplex Assays



- xTAG Respiratory Viral Panel (Luminex Corp)
 - Influenza A and B (H1, H3), parainfluenza 1-3, RSV (A and B), rhinovirus, adenovirus, hMPV (no coronaviruses)
- MultiCode Respiratory assay (EraGen)
 - Influenza A/B, parainfluenza 1-4, RSV (A/B), rhinovirus, hMPV, adenovirus, coronaviruses
- ResPlex II (Geneco, QIAGEN)
 - Flu A/B, PI 1-4, RSV, hMPV, rhinoviruses, enteroviruses, SARS (no other coronaviruses or adenovirus)



rRT-PCR Flu Panel

- Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panel.
- Approved by FDA in October.
- Can detect common strains of seasonal influenza and H5 (avian) flu.
- Will be used starting this flu season



Do We Really Need to Know?

- Any respiratory infection that develops after admission is problematic and should be explored.
- We don't necessarily need to know the etiology to implement control measures:
 - Isolation
 - Surveillance
 - Good hand hygiene



Knowledge is Good

- But, knowing the specific etiology can help direct control measures:
 - Should you be focusing on droplet or contact precautions or both?
 - Would not want to cohort kids with different infections together.
 - Antivirals and/or vaccines might be useful



Another Important Reason to Know

- Making diagnoses in outbreak investigations can help identify the emergence novel pathogens
 - Adenovirus
 - SARS
 - Avian influenza



Common Things Are Still Common



- Most healthcare outbreaks are still caused by influenza and RSV.
- DFA still plays a key role in rapid diagnosis for implementing infection control.
- However, we now have more options available to us in cases where DFAs are negative.
- NAT tests will also help us diagnose outbreaks that we could not before



Rapid MRSA Diagnostics

- Increased interest in and use of MRSA screening.
 - Some driven by healthcare facilities
 - Some driven by legislatures
- This interest has led to a huge effort to develop new diagnostic tests for MRSA.
- These tests will also have implications for investigating MRSA outbreaks.



New MRSA Diagnostics



- Culture based
 - Selective and chromogenic agars
- Molecular



Culture Based MRSA Diagnostics

- Old

- Mannitol salt agar (selective for *S. aureus*) with cefoxitin (selective for MRSA)

- Newer

- Chromogenic agars where MRSA colonies turn a different color from other bacteria

Direct Culture (Selective/Differential Agar)

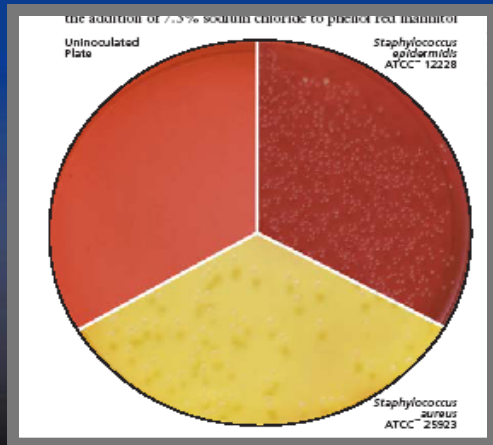
MRSA Spectra



CHROMagar MRSA



MRSA Select



Mannitol Salt Agar w/Cefoxitin



Incubation Times for Direct Culture

Percent of MRSA recovered after 24 h incubation

Agar	% MRSA Detected	Reference
CHROMagar	84	JCM. 2008. 46:350
Select	96	JCM.2006.44:4 561
Spectra	95.4	Package Insert
MS w/cefoxitin	77	JCM.2006.44:4 561



Culture based methods: Pros and Cons

- Pros:
 - Don't require new equipment
 - Less expensive
 - Result in an isolate for typing, if needed
 - Gold standard because the organism actually has to grow
- Cons:
 - Slower
 - Might be less sensitive than molecular?



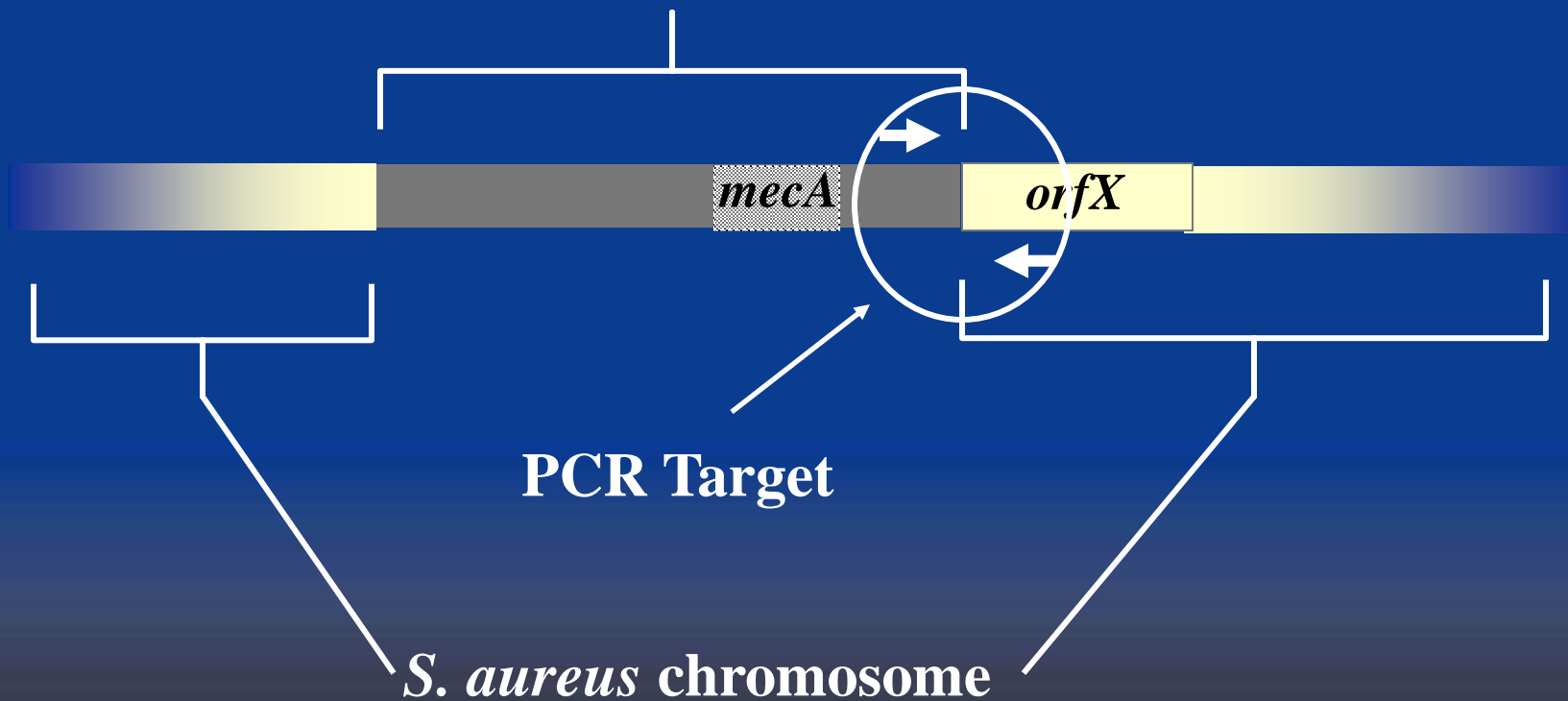
Molecular MRSA Diagnostics

- Huge growth in this area
- Available tests are PCR based and rely on amplification of a target sequence unique to MRSA, community or healthcare associated.

PCR: Target Sequence



SCCmec element (I, II, III, IV, V)



Performance Characteristics: GeneOhm MRSA vs. Xpert MRSA

PCR assay performance compared to reference culture method

Method	Sensitivity	Specificity
Xpert MRSA	86.3%	94.9%
GeneOhm MRSA	83.3%	94.4%
Direct Culture	82.9%	100.0%

~~7 lab study, 1074 specimens, 19.6% MRSA prevalence~~

Data from Xpert MRSA 510(K) Decision Summary

PCR Test Performance for Specimens from Different Body Sites

IDI-MRSA (PCR) compared to broth-enriched culture

Body Site	MRSA Prevalence*	Sensitivity	Specificity
Nose	13.5%	88.5 %	94.6 %
Groin	11.4%	81.8 %	91.8 %
Combined Nose-Groin	16.1%	87.1 %	94.4 %

*Based upon broth enriched culture
EJ Bishop, et al. JCM. 2006. 44: 2904



Molecular Methods: Pros and Cons



- Pro:
 - Results can be obtained quickly- performance time for the assays is less than 2 hours
 - Might be more sensitive than culture
- Con:
 - More expensive and require special equipment
 - Requires additional training for lab staff
 - Are these tests too sensitive?
 - How much faster are they?

Is PCR More Sensitive?



- Study expanded “true positive” PCR definition to includes MRSA history/risk factors
 - PCR + specimens which grew MRSA in culture
 - PCR +, culture negative for MRSA, but history of MRSA within the last year or antistaph antibiotics in the prior month

Method	Sensitivity
GeneOhm	98.2%
Direct Culture (CNA)	75.3%
Broth Enriched Culture	84.8%



Is PCR Too Sensitive?

- Studies have shown PCR positive, but cultures negative in 9-15% of cases.
 - Might be enhanced detection of very low organism burden?
- But, have also shown PCR positive, but cultures grow MSSA in 15-26%.
 - Might be really detecting “hidden” MRSA
 - Might be actually detecting MSSA that carry *SCCmec* that has lost *mecA* gene.



PCR Assays: Costs

	GeneOhm MRSA	Xpert MRSA
Reagent cost per specimen	\$25	\$42
Instrumentation costs	Smart Cyclor (16 modules) \$34,399	GeneXpert (8 module) \$120,750

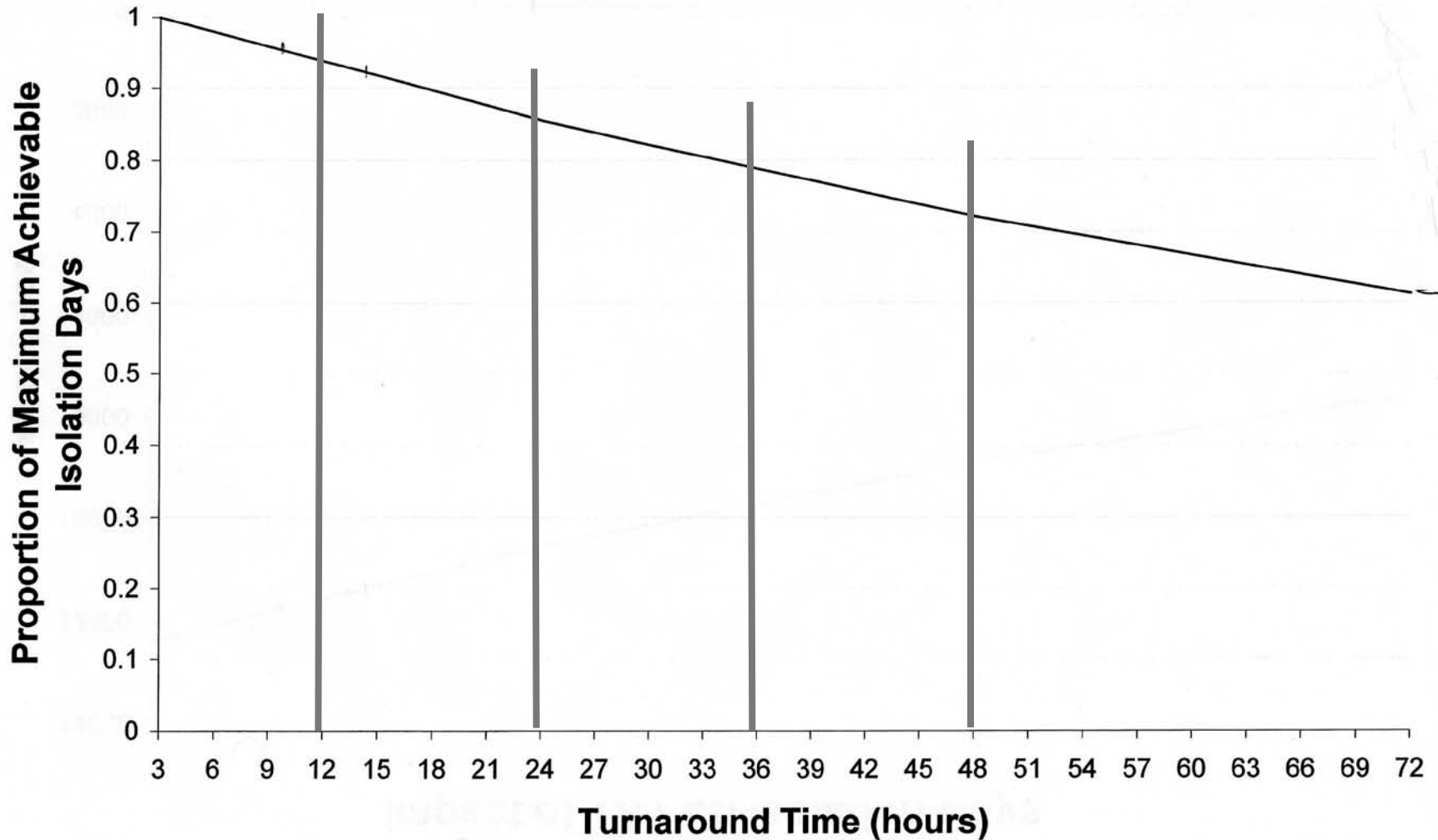
Selective agars: ~ \$3-5 dollars (less for mannitol salt)



Molecular Detection- The Need for Speed

- If the goal of screening is to get colonized patients into isolation to reduce transmission risks, then it would follow that the faster we get the results, the more effective our isolation strategies will be.
- This would also certainly apply to outbreaks.

Impact of TAT on Isolation Days




Northwestern Evaluation – Courtesy of Tom Thomson




How Fast Are Molecular Tests?

- Assays take less than 2 hours to perform.
- But, it depends on how often samples are being run
 - If batched once a day, it could be 24 hours to get a result
- Studies have reported ranges from 4 hours to 24 hours to lab turn around time.



But Remember Time to do the test \neq Time to Isolation



- Specimen Collection
- Specimen Transport
- Accessioning
- **Testing**
- Reporting
- Communicating the Report
- Implementing Interventions



Molecular Tests on the Horizon for MRSA

- Current rapid tests are approved for use for surveillance swabs.
- Studies are underway looking at these technologies for diagnosing MRSA :
 - Directly from blood cultures
 - One test already approved for this
 - Directly from wound samples



Other Molecular Tests in Development

- Rapid detection assays for:
- Vancomycin resistant *Enterococcus*
- *Clostridium difficile*



Conclusions

- Diagnostic and typing methods remain important tools in outbreak investigations.
- New diagnostics are improving both our ability to detect pathogens and the speed with which we can detect them.
- But, these tools must be paired with sound epidemiologic investigations!



Thanks!

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The findings and conclusions in this presentation are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention

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