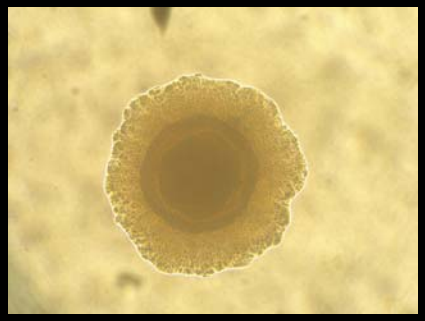
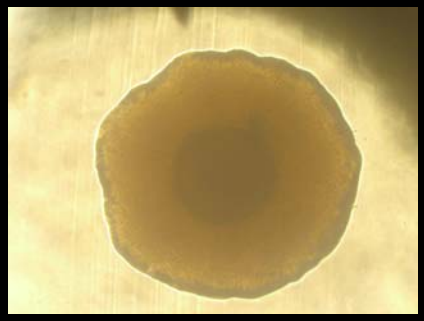
MOLECULAR DETECTION OF ANTIMICROBIAL RESISTANT PATHOGENS





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Summary of talk

- Will describe a recently published study
- Discuss additional ongoing project related to antimicrobial resistance

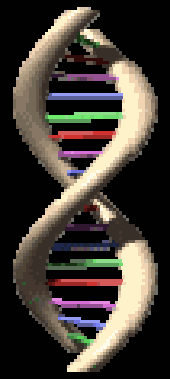
CLINICAL MICROBIOLOGY RESEARCH OBJECTIVE

- Develop new molecular diagnostic tools and demonstrate how these can be used to improve the rapid detection of drug resistant pathogens

- **Long-term Goal:** Reduce and control antimicrobial resistance

WHY MOLECULAR MICRO?

- Faster time to results
  - ✓ 2 hr vs. 48-72 hrs (or weeks in some cases)
- Expedited institution of Infection Control precautions
- Reduction
  - ✓ Nosocomial infection rates
  - ✓ Hospital length of stay
  - ✓ Spread of resistance into the environment/community
  - ✓ Healthcare costs
- Improved clinical outcomes



Original Research

Prevalence of Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* in Pregnant Women

Katherine T. Chen, MD, MPH, Richard C. Huard, PhD,* Phyllis Della-Latta, PhD, MSc, and Lisa Saiman, MD, MPH*

Editorial

Emerging Infections in Obstetric and Gynecologic Practice

THE INTERDISCIPLINARY TEAM

- CLINICAL MICROBIOLOGY
  - ✓Dr. Phyllis Della-Latta
- PEDIATRIC INFECTIOUS DISEASES
  - ✓Dr. Lisa Saiman
- OB/GYN + EPIDEMIOLOGY
  - ✓Dr. Katherine Chen

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

- **Important points:**
  1. Almost 60% of all healthcare-associated *S. aureus* infections are due to MRSA
  2. High morbidity and mortality and excess hospitalization costs are associated with MRSA infections
  3. MRSA colonization of persons in the hospital and the community acts as a reservoir for continued dissemination
  4. Colonized persons are at higher risk of infection by these strains

GOAL

Determine the carriage of CA-MRSA in a cohort of pregnant women from our local community.

- **Interdisciplinary Project:**
  - ✓ **Prospective surveillance study for methicillin-sensitive *S. aureus* (MSSA) and MRSA in prenatal group B *Streptococcus* (GBS) screening cultures**
  - ✓ **Standard-of-care protocols for GBS screening**
  - ✓ **Finalized cultures then evaluated for *S. aureus* by looking for growth on solid culture media**
  - ✓ **Identified strains were further characterized – routine microbiology and molecular techniques**

- **Overall,**
  - ✓ 2963 vaginal/rectal GBS screening cultures
  - ✓ 508 *S. aureus* (17.1%)
  - ✓ 14 MRSA (0.5%)
- **SCC*mec*-typing**
  - ✓ 1 HA-MRSA (SCC*mec* type II).
  - ✓ 13 CA-MRSA (SCC*mec* type IV and type V)

- **Relevance:**
  - ✓ First to estimate the vaginal/rectal carriage of MSSA in pregnant women using prenatal GBS screening cultures
  - ✓ First study to find vaginal/rectal MRSA colonization in women (pregnant or otherwise)
- **Future questions to address:**
  - ✓ Not known if vaginal/rectal MRSA carriage is associated with any risks to the mother or the neonate
  - ✓ MRSA in the NICU is a big worry – should we be screening the mothers for vaginal/rectal MRSA? What to do if MRSA colonization is found?

Carbapenem-Resistant *Klebsiella pneumoniae*

- *K. pneumoniae*:
  1. Gram negative rod – pneumonia, blood stream, wound, and urinary tract infections
  2. Ranks 4th as a cause of HA pneumonia in the USA
  3. Carbapenems (imipenem, meropenem, ertapenem) an important treatment option – potent and broadly active
  4. KP resistant to carbapenems emerging since yr 2000 in NYC and upstate NY
  5. Many fatalities
  6. KPC one mechanism
  7. KPC = *K. pneumoniae* carbapenemase

Carbapenem-Resistant *Klebsiella pneumoniae*

- **Important points:**
  8. **KPC2, KPC3 – plasmid borne genes**
  9. **Have been identified in other enterobacteriaceae species (*E. coli*, *Salmonella*, *Enterobacter*)**
  10. **Spreading west – identified in MI, OH, AZ (outbreak)**
  11. **Columbia, France, China – sporadic cases**
  12. **Most KPC+ KP also resistant to cephalosporins, penicillins, aminoglycosides**
  13. **Reduced permeability because of porin loss may potentiate carbapenem resistance**

Carbapenem-Resistant *Klebsiella pneumoniae*

- **Important points:**
  14. **Relying on old drugs like polymyxin B and colistin to treat – pan-resistant strains are emerging**
  15. **35-40% of clinical KP isolates are KPC +ve at some institutions**
  16. **Identification of KPC-mediated resistance is often missed by initial routine (automated) microbiologic testing!!!**
  17. **These require secondary agar-based susceptibility tests to catch**

GOAL

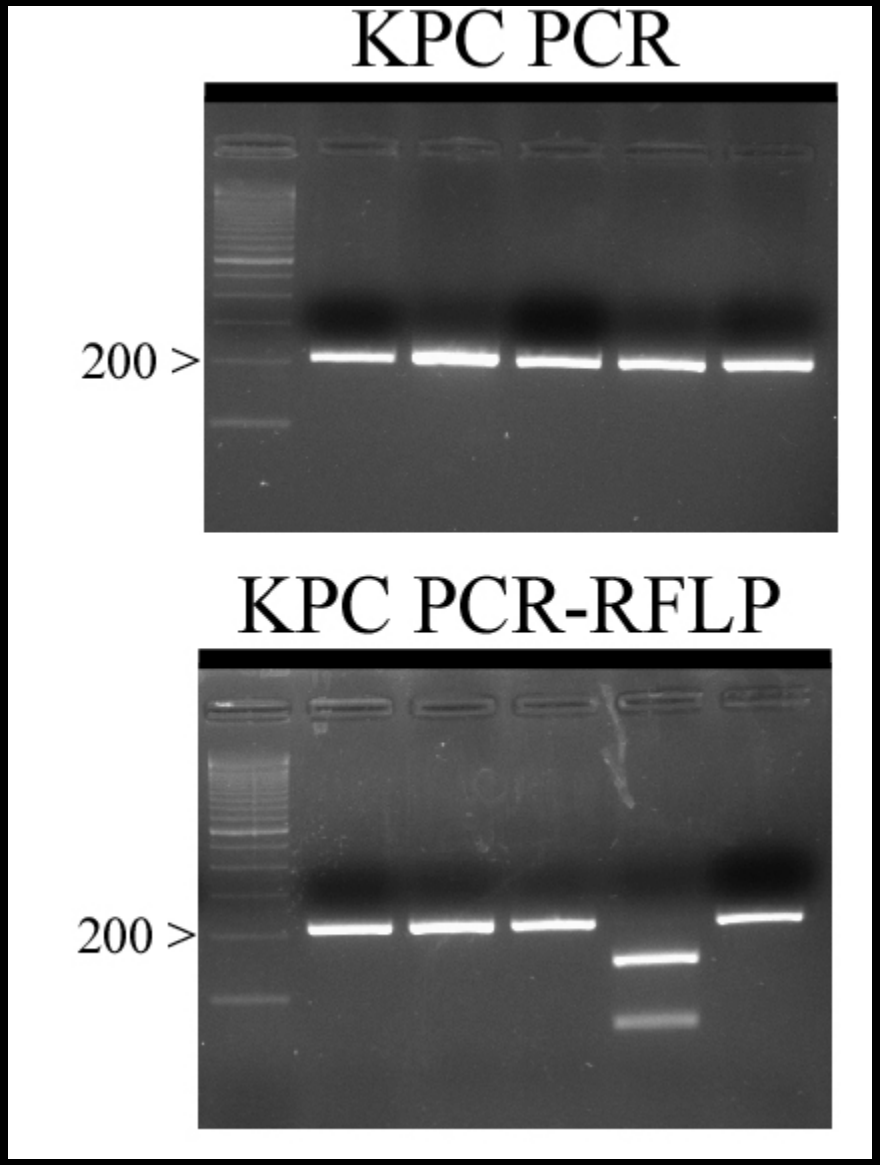
Develop a simple first-generation test identify the presence of KPC and to characterize the KPC-types of our patient population.

- **Molecular Approaches:**
  - ✓ **Real-time PCR: difficulties in type-specific probe design**
  - ✓ **Success using a restriction fragment length polymorphism (RFLP) approach**

KPC RFLP Approach

1. Regular PCR amplification a KPC gene fragment
2. Cut the product with a specific enzyme (*Rsa* I) that recognizes the difference between KPC2 and KPC3
3. Run on a gel and look for difference is band sizes – one band (no cut) = KPC2, two bands (one cut) = KPC3

KPC RFLP Approach



- **Saw both KPC2 and KPC3**
  - ✓ **Confirmed by sequence analysis.**
  - ✓ **KPC2 > KPC3**
  - ✓ **Novel observation**
  - ✓ **Expanded evaluation now underway**

- **Relevance:**
  - ✓ Carbapenem-resistant KP are spreading and can be missed so labs need better tests to identify
  - ✓ Epidemiological tool
- **Future questions to address by an interdisciplinary approach:**
  - ✓ Clinical relevance of infection with different KPC-types?
  - ✓ Estimate the current prevalence and track changes over time?
  - ✓ Control spread with accurate detection?
  - ✓ Selection power of other antibiotics?
  - ✓ Origin/natural reservoir?

THANKS