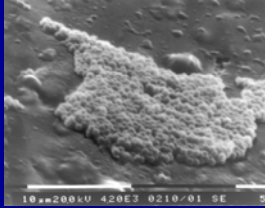


## Staphylococcus aureus

- Significant human pathogen.
  - SSTI
  - Biomaterial related infections
  - Osteomyelitis
  - Endocarditis
  - Toxin mediated diseases
    - TSST
    - Staphylococcal enterotoxins



10 μm 20.0 kV 4.20E3 0.210/01 SE 5

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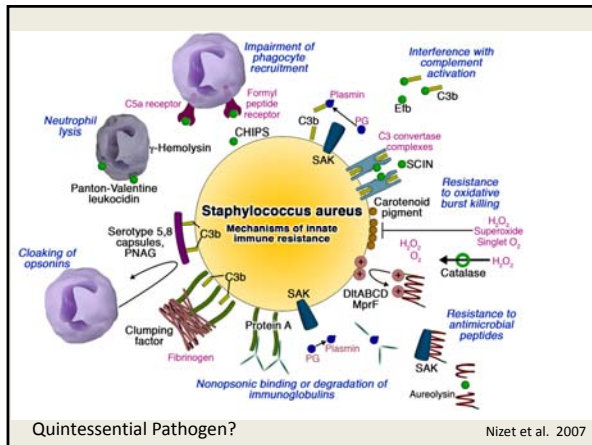
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## Goals

- What are the differences between the MRSA of 2008 and 1988?
  - What is a strain?
  - USA 400, 300
  - Clonal expansion
  - New virulence factors?
- What are new technologies used to detect MRSA in the clinical microbiology laboratory?

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## What is a strain of a bacterial species?

- Through phenotypic or genotypic methods, one can differentiate between members of the same species— these different isolates are called unique strains.
  - Uropathogenic *E. coli* isolates vs. *E. coli* O157:H7
- Methods
  - Phenotypic methods (e.g. carbohydrate fermentation)
  - Epidemiological methods (e.g. Pulsed-field gel electrophoresis [PFGE])
  - Population biology methods (e.g. Multi-locus sequencing typing (MLST), VNTR analysis, whole genome sequencing)

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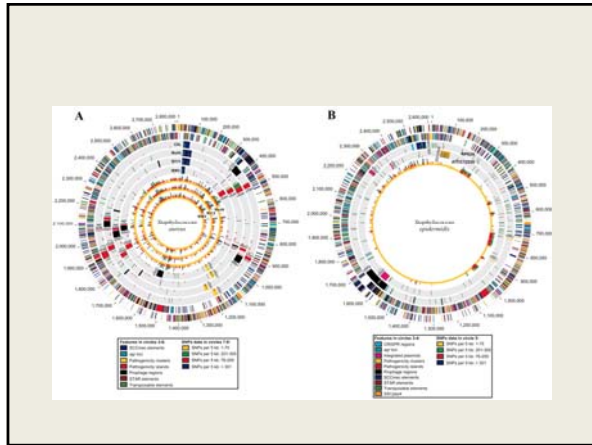
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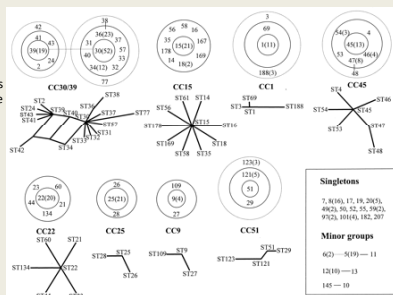
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## *S. aureus* lineages (e.g. clonal backgrounds, genotypes, strains)

MLST-defines clonal complexes and/or sequence types



Feil et al. J. Bact. 2003

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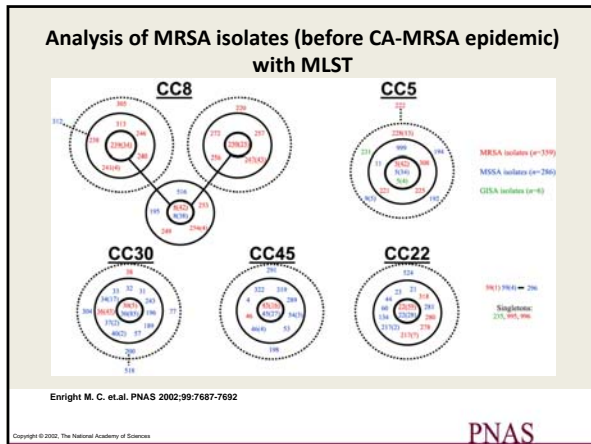
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**1999 MMWR**

- Four Pediatric deaths in Minnesota and North Dakota caused by CA-MRSA.
  - No known MRSA risk factors
  - Susceptible to non- $\beta$ -lactam antibiotics
  - Pediatric patients

CDC. 1999. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997-1999. *Morb. Mortal. Wkly. Rep.* 48:707-71

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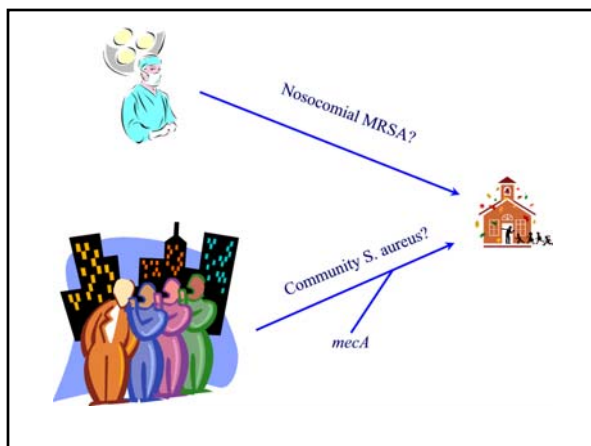
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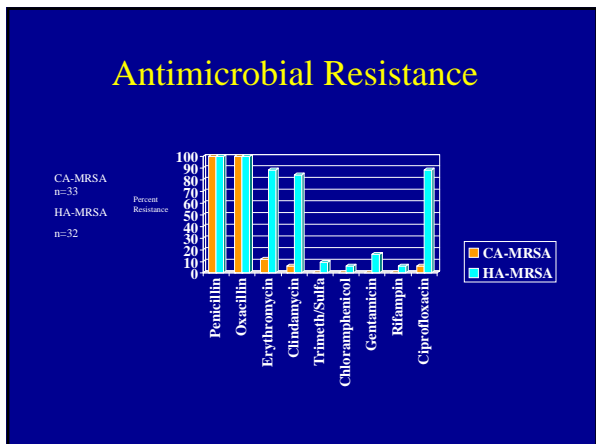
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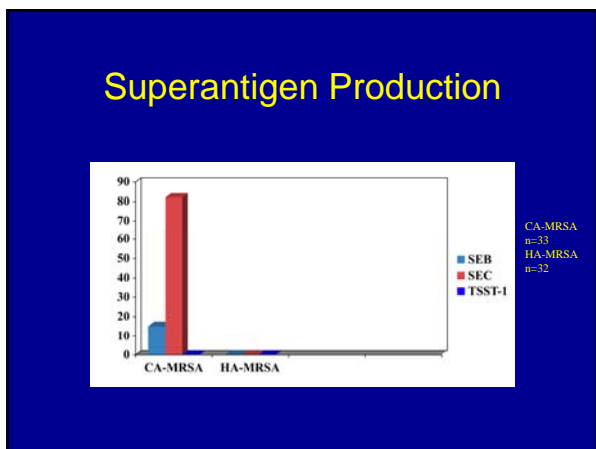
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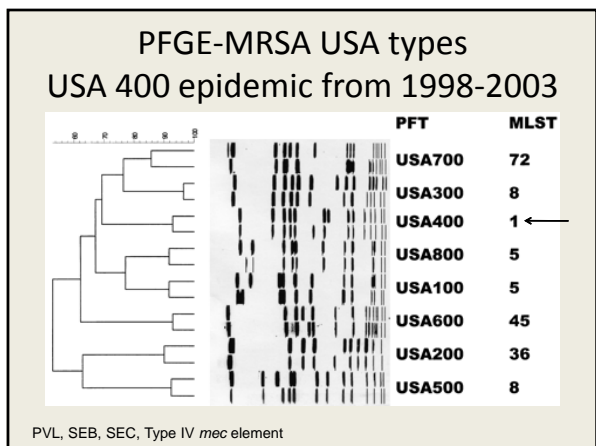
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Prevalence of CA-MRSA ~2003-present

Methicillin-Resistant *S. aureus* Infections among Patients in the Emergency Department  
 Gregory J. Moran, M.D., Anusha Krishnasdasan, Ph.D., Rachel J. Gorwitz, M.D., M.P.H., Gregory E. Fosheim, M.P.H., Linda K. McDougal, M.S., Roberta B. Carey, Ph.D., and David A. Talan, M.D., for the EMERGENCY ID Net Study Group\*

- Survey of 11 EDs throughout US in Aug 2004
- 422 pts with skin & soft tissue infection
- 320/422 (75%) caused by *S. aureus*
- MRSA 59% (15% - 74%), USA300 strain 97%
  - KC 74%; Atlanta 72%, Charlotte NC 68%, New Orleans 67%, Albuquerque 60%, Phoenix 60%, Philadelphia 55%, Portland OR 54%, Los Angeles 51%, Minneapolis 39%, New York 15%

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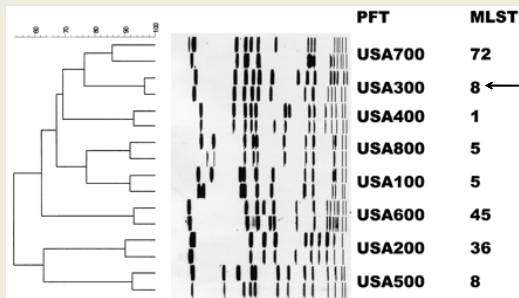
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PFGE-MRSA USA types  
 USA 300 epidemic from 2003-present



PVL, SEB, SEC, Type IV *mec* element

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- USA 300 MRSA most prominent *S. aureus* lineage isolated in the US.
  - Not restricted to community
  - Not as susceptible to non-β-lactam antibiotics. Becoming resistant to mupirocin, clindamycin and SXT
- What is so special about USA300?
  - Nobody knows

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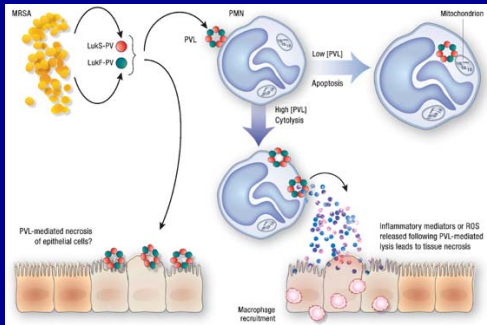
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### Panton-Valentine Leukocidin-USA300 and 400



Boyle-Vavra & Daum, Lab Invest, 2007

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### Panton Valentine Leukocidin

- Data to suggest that PVL has a significant role in pathogenesis of necrotizing pneumonia
- However, newer data suggests that PVL has a complementary role and is NOT the major virulence factor associated with USA300 pathogenesis (SSTI or pneumonia).

Labandiera-Rey et al. Science 2007 Feb 23;315(5815):1130-3  
 Bubeck-Wardenburg. Nature Medicine 13, 1405 - 1406 (2007)  
 Voyich et al. J Infect Dis. 2006 Dec 15;194(12):1761-70

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### Other unique factors that USA300 carries

- Increased  $\alpha$ -toxin production.
  - $\beta$ -barrel structure. HLA forms pores in lymphocytes, macrophages, alveolar epithelial cells, pulmonary endothelium and erythrocytes. *hla*-negative mutants are avirulent in mouse model of pneumonia. Antibody to  $\alpha$ -toxin protective in pneumonia model. J Exp Med. 2008. Feb 18; 205(2):287-294.
- Phenol soluble modulins production (PSM)
  - Recruit, activate, and lyse human neutrophils. Nature Medicine. 2007. 13:1510-1514.
- ACME (Arginine catabolic mobile element) Pathogenicity Island
  - Possibly involved in skin colonization
  - ACME deficient USA300 are less virulent in bacteremia model. JID. 2008; 197:1523-30.

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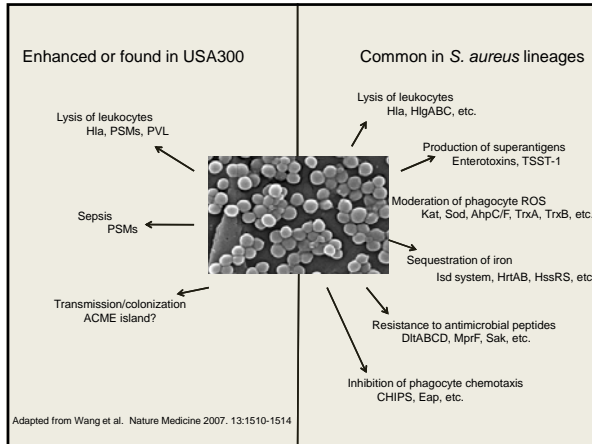
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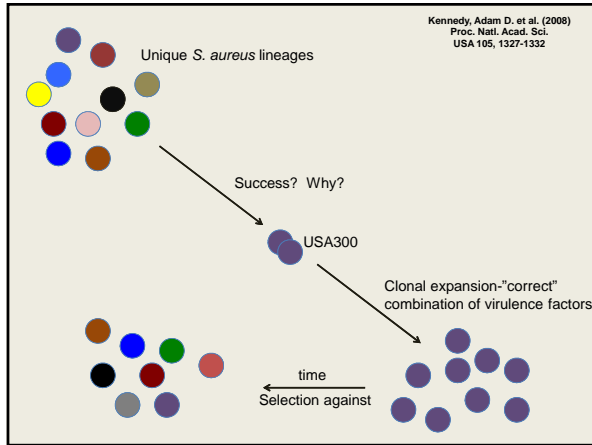
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**How does the Clinical Microbiology Laboratory detect MRSA from nasal swabs?**

- Mannitol salt agar with cefoxitin
  - Least sensitive and most time consuming
- Chromogenic agar-Detects specific enzymes within *S. aureus* ( $\beta$ -glucosidase or a phosphatase) plus chromogenic cephamycin.
  - Low sensitivity (~75%), high specificity at 24 hours
  - increased sensitivity at 48 hours

JCM 2008: 46:1577-1587

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## Molecular Assays

- Due to increased MRSA screening in hospitals, Microbiology laboratories need to invest in methodologies that decrease turn around time.
- PCR-based assays (same day results)
  - Demonstrated to decrease MRSA transmission incidence (J. Hosp. Infect. 2007. 65:24-28)
  - IDI-MRSA/GeneOhm MRSA
  - GeneXpert MRSA assay
    - Both yield increased sensitivity (~85 to 100%)
    - GeneXpert allows for single sample testing without batching.

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## Conclusions

- *S. aureus* USA300 is an epidemic in the United States.
- Contains a repertoire of virulence factors that allow it to effectively colonize and cause wide variety of disease manifestations.
- Due to increased screening of patients to detect MRSA colonization, microbiology laboratories need to develop expertise in PCR technologies.

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