Methicillin-resistant *Staphylococcus aureus* (MRSA) Virulence & Immune Evasion

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The Modern Antibiotic Era

Discovered Penicillin in 1928
Inhibited growth of *S. aureus*

Sir Alexander Fleming

*Staph aureus* Drug Resistance & Epidemics

First use of Penicillin, 1941

Sir Ernest Chain 1940 1950 1960 1970
Sir Howard Florey 1980 1990 2000
Emergence of Antibiotic Resistance

Naturally Occurring Resistant Bacteria

Susceptible Bacteria

Lateral Gene Transfer

DNA with Resistance Gene(s)

Antibiotics

Enrichment for New Resistant Bacteria

Modified from the Centers for Disease Control & Prevention

Typical Methicillin-Resistance Cassettes

a SCCmecII

<table>
<thead>
<tr>
<th>orfX</th>
<th>ccrAB</th>
<th>Tn554</th>
<th>mec</th>
<th>IS431</th>
</tr>
</thead>
</table>

b SCCmecIV

| orfX | ccrAB | mec | IS431 |

DeLeo & Chambers, Nat Rev Microbiol, 2009

Staphylococcus aureus:
Drug Resistance & Epidemics

Penicillin, 1941
Penicillin-resistant S. aureus
Pandemic S. aureus
Methicillin, 1959
Methicillin-resistant S. aureus (MRSA)
1st MRSA outbreak in USA
Worldwide spread of MRSA


Adapted from DeLeo & Chambers, J Clin Invest, 2009
**Epidemic Healthcare-Associated S. aureus**

Cost to the U.S. in 2003 was $14.5 billion\(^1\) & mostly MRSA

Severe MRSA infections occur in ~31 out of every 100,000 people. ~20% cause death\(^2\)

Antibiotic resistance *per se* does not confer enhanced virulence—it is a problem for treatment

\(^1\) Noskin et al., *Clin Infect Dis*, 2007
\(^2\) Klevens et al., *JAMA*, 2007

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**Staphylococcus aureus: Drug Resistance & Epidemics**

Penicillin, 1941

Penicillin-resistant *S. aureus* (PRSA)


Adapted from DeLeo & Chambers, *J Clin Invest*, 2009

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**Staphylococcus aureus: Drug Resistance & Epidemics**

Penicillin, 1941

Penicillin-resistant *S. aureus*

Pandemic *S. aureus*

Methicillin, 1959

Methicillin-resistant *S. aureus* (MRSA)

1st MRSA outbreak in USA

Worldwide spread of MRSA

Community MRSA


Adapted from DeLeo & Chambers, *J Clin Invest*, 2009
Global Distribution of Community-Associated MRSA (CA-MRSA)

DeLeo, Otto, Kreiswirth, Chambers, Lancet, 2010

CA-MRSA: Epidemic in the United States

Causes disease in otherwise healthy individuals

Predominantly skin and soft tissue infections (~75-95%), but invasive disease is severe

The most prevalent CA-MRSA isolates in the United States are USA300 (Los Angeles County clone, LAC). First to emerge in the US were USA400 (MW2).

Collaborative Network to Study MRSA

UCSF
RML, NIAID
Columbia U
PHRI
U Chicago
Texas Medical Center
U New Mexico
Prominent CA-MRSA are Highly Virulent

Hospital

Mouse survival (%)

Community

Time after infection (h)

USA300 Causes Severe Dermonecrosis in the Mouse Skin Infection Model

Dermonecrosis

Abscess

Neutrophils & cell debris

Adapted from Kennedy et al., J Infect Dis, 2010

Neutrophil Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Defect</th>
<th>Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chediak-Higashi Syndrome</td>
<td>LYST (CHS1)</td>
<td>S. aureus, GDS</td>
</tr>
<tr>
<td>Chronic Granulomatous Disease</td>
<td>CYBA, CYBB, NCF1, NCF2</td>
<td>S. aureus, B. cepacia, Serratia marcescens, Aspergillus spp., Nocardia spp.</td>
</tr>
<tr>
<td>Hyper-IgE Syndrome (Job’s Syndrome)</td>
<td>STAT3 (STAT3)</td>
<td>S. aureus</td>
</tr>
<tr>
<td>Leukocyte Adhesion Deficiency</td>
<td>INTR2 (CD18) CHO fucosylation</td>
<td>S. aureus, G- bacteria</td>
</tr>
<tr>
<td>Myeloperoxidase Deficiency</td>
<td>MPO (MPO)</td>
<td>Candida albicans w/ diabetes mellitus</td>
</tr>
<tr>
<td>Neutrophil-Specific Granule Deficiency</td>
<td>CEBPE (C/EBPα)</td>
<td>S. aureus, S. epidermidis</td>
</tr>
<tr>
<td>Severe congenital neutropenia (Kostmann’s syndrome)</td>
<td>ELA2 (elastase) HAX1, CSF3R</td>
<td>S. aureus, many path.</td>
</tr>
</tbody>
</table>
Neutrophil Phagocytosis & Microbicidal Processes

Kobayashi & DeLeo, WIREs Syst Biol Med, 2009

Two Possible Outcomes of PMN-Bacteria Interactions

Kobayashi & DeLeo, WIREs Syst Biol Med, 2009
Common *S. aureus* Virulence Factors

- Lysis of leukocytes: Hla, HlgABC, LukDE, PSMs, PVL, etc
- Production of superantigens: Enterotoxins, e.g., TSST-1
- Moderation of phagocyte ROS: Kat, Sod, AhpCF, TrxA, TrxB, etc
- Sequestration of iron: Isd system, HtrAB, HssRS, etc
- Sequestration of antibody: Protein A (Spa) and Sbi
- Resistance to antimicrobial peptides: DltABCD, MprF, Sak, etc
- Inhibition of phagocyte chemotaxis: CHIPs, SCIN, Eap, etc

Adapted from Wang et al., Nat Med, 2007.

Phagocytosis of USA300 by Human PMNs in Blood

Phagocytosis of USA300 or USA400 is rapid and activates the chief microbicidal processes in human neutrophils.

There is significant survival of USA300 and USA400 after phagocytosis (35-50%).

Survival of *S. aureus* Within PMNs

PMN Lysis after Phagocytosis

Human PMN & MW2 at 0.5 h
How Does CA-MRSA Fit into the Model?

Kobayashi & DeLeo, WIREs Syst Biol Med, 2009
Rapid Neutrophil Lysis Following Phagocytosis

USA300-Mediated Neutrophil Lysis
Putative or Proven Leukotoxins of USA300

**Gene:**
- hlgA
- hlgB
- hlgC
- lukD
- lukE
- PVL
- Hla

**Protein:**
- HlgABC
- LukDE
- PVL
- Hla (α-toxin)

Panton-Valentine Leukocidin (PVL)

Pore-forming leukotoxin with known cytolytic activity toward myeloid cells

P.N. Panton and F.C.O. Valentine
_Lancet_, 1932, March 5, p 506-508.

PVL genes are associated with many strains that cause CA-MRSA infections

Panton-Valentine Leukocidin Does Not Contribute to Human Neutrophil Lysis After Phagocytosis

Voyich et al., _J Infect Dis_, 2006
PVL & Skin Infection in the Mouse

USA400
MW2
MW2Δpvl
USA300
LAC
LACΔpvl
1 cm
1 cm

Voyich et al., J Infect Dis, 2006

PVL and Virulence

Most data from experimental & human clinical studies suggest PVL is not the main determinant of CA-MRSA virulence

Might contribute to virulence under unique conditions

PSMα Promotes Skin & Soft Tissue Infection

LAC (USA300)

LACΔpsm-α

Adapted from Wang et al., Nat Med, 2007
**Alpha-Hemolysin (Hla) Contributes to Severity of USA300 (LAC) Skin Infection in the Mouse**

- wild-type
- Δhla
- Δhla::phla

Kennedy et al., *J Infect Dis*, 2010

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**Active Immunization Against Hla Moderates Severity of Skin Infections**

- LAC (USA300) – Day 5

- Sham
- Immunized

Kennedy et al., *J Infect Dis*, 2010

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**Putative or Proven Leukotoxins of USA300**

- Genes:
  - hlgA
  - hlgB
  - hlgC
  - lukS
  - lukF
  - lukE
  - lukD
  - lukC
  - lukB
  - lukA
  - lukI
  - lukH
  - lukG

- Proteins:
  - HlgABC
  - LukDE
  - PVL
  - Hla (α-toxin)

lukS lukF
LukGH Contributes to PMN Lysis After Phagocytosis

![LukGH Contributes to PMN Lysis After Phagocytosis](image)

Ventura et al., PLoS ONE, 2010

Rabbit Model of *S. aureus* Skin Infection

![Rabbit Model of *S. aureus* Skin Infection](image)

SD Kobayashi et al., unpublished

PVL and PSMα & Skin Infection in the Rabbit

![PVL and PSMα & Skin Infection in the Rabbit](image)

SD Kobayashi et al., unpublished
Conclusions

Phagocytosis of USA300 or USA400 is rapid and fully activates neutrophils; however, there is significant pathogen survival.

The enhanced ability of CA-MRSA strains to circumvent killing by neutrophils is linked to enhanced virulence.

Neutrophil lysis following phagocytosis of *S. aureus* is likely a component of pathogenesis, but the mechanism remains to be determined.

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