Session 1
Alternatives to Antibiotics: Lessons from Nature

Antimicrobials in animal health – Lessons from nature

Frank Blecha
Kansas State University
Manhattan, Kansas
Alternatives to Antibiotics

*Begin at the End*

- Is there a need?
is for Outbreak

When a drug-resistant salmonella struck a Vermont farm, health officials knew it might be just the beginning

Troubled paradise

Troubled paradise

U.S. News

November 24, 1997
Alternatives to Antibiotics

Begin at the End

• Is there a need?
• Does it work?
• Is it cost effective?
• Will it be accepted?
Host Defense Against Microbes

Physical barriers

Cells

Antimicrobial peptides

Adapted from Ganz T, 2003
What are Antimicrobial Peptides?

PMNs
Neutrophils
Host Defense Against Microbes

Antimicrobial peptides:

PMN

Neutrophils

Bainton, 1992
AMP Classification

Cathelicidins  Defensins

alpha  beta  theta
Cathelicidins

**EXON 1**          **EXON 2**    **EXON 3**            **EXON 4**

**PRE**          **PRO (Cathelin)**          **MATURE PEPTIDE**

5’-UTR          29-30 aa          94-114 aa          12-79 aa          3’-UTR

Porcine     Bovine        Ovine          Lapine       Human       Equine       Murine
PR-39        Bac-5          SMAP-29        CAP-18        LL-37/        eCATH 1-3     CRAMP
PG 1-5       Bac-7          Bac-7.5         p-15s          hCAP-18
PF 1-2       BMAPs          Dodecapeptide
PMAPs        Indolicidin    Dodecapeptide

**3’-UTR**
Beta Defensins

EXON 1 INTRON EXON 2

PRE PRO MATURE PEPTIDE

5'-UTR 20-29 aa 2-8 aa 36-42 aa 3'-UTR

Porcine Bovine Avian Murine Ovine Caprine Human
pBD-1 TAP GAL 1-2 mBD 1-6 sBD 1-2 gBD 1-2 hBD 1-4
LAP THP 1-2 rBD 1-2

EBD BNBD 1-13

Sang et al., Mamm. Genome, 2006, 17:332
Why AMPs?

• Potential for novel antimicrobials
• Active against a broad spectrum of microbes
• Difficult for microbes to develop resistance
AMP Mechanism of Action

Adapted from Radek and Gallo, Semin Immunopathol 2007, 29:27-43
AMP or Host Defense Peptide

In Vivo Activity?
HD6 nanonets confer mucosal immunity

Ouellette and Selsted, Science 2012;337:420-421
Lessons from the Pig
PR-39, an Amphipathic Peptide

RRRPRPPYLPRLPRPPPPFPPRPLPPRIPPGFPPRFPPRFP

Proline = 48.7%  Arginine = 25.6%

Basic, Neutral Polar, Nonpolar
## PR-39 and Analogues

<table>
<thead>
<tr>
<th>PR-14&lt;sub&gt;(1-14)&lt;/sub&gt;</th>
<th>RRRPRPPPYLPRPRPRP</th>
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<tbody>
<tr>
<td>PR-16&lt;sub&gt;(11-26)&lt;/sub&gt;</td>
<td>RPRPPPFFPRLPPRI</td>
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<tr>
<td>PR-15&lt;sub&gt;(25-39)&lt;/sub&gt;</td>
<td>RIPPGFPPRFPFRFP</td>
</tr>
<tr>
<td>PR-27&lt;sub&gt;(13-39)&lt;/sub&gt;</td>
<td>RPPPFFPPRLPPRIPPGFPFRFP</td>
</tr>
<tr>
<td>PR-39</td>
<td>RRRPRPPPYLPRPRPPPPFFFFPPRLPPRIPPGFPFRFP</td>
</tr>
</tbody>
</table>

| PR-26<sub>(1-26)</sub> | RRRPRPPPYLPRPRPPPPFFFFP PRLPPRI |
| PR-19<sub>(1-19)</sub> | RRRPRPPPYLPRPRPPPPPFFP |
| PR-23<sub>(4-26)</sub> | PRPPYLPRPRPPPPFFFFPRLPPRI |
| PR-26<sub>G(1-26)</sub> | RRRPRPGPYLPRPPPPFFFFPRLPPRI |
Minimal Inhibitory and Bactericidal Concentrations of PR-39 & PR-26

Shi et al., Antimicrobial Agents Chemotherapy 1996, 40:115-121
Inhibition of *Salmonella* Typhimurium Invasion

**Graph:**
- Y-axis: Viable bacteria (x1000)
- X-axis: Incubation time (min)
- Control, PR-39, PR-26

*Shi et al., Antimicrobial Agents Chemotherapy 1996, 40:115-121*
Increased Resistance to *Salmonella Typhimurium*

![Graph showing survival (%) over day postchallenge](image)

- **Survival (%)**
- **Day postchallenge**

*Shi et al., Antimicrobial Agents Chemotherapy 1996, 40:115-121*
pBD-1 Protects against *B. pertussis*

Treated with pBD-1

Non-treated

Days Post Challenge

4

Bacterial Resistance to AMP?

Adapted from Radek and Gallo, Semin Immunopathol 2007, 29:27-43
Alternatives to Antibiotics

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- • Is there a need?
+/- • Does it work?
- • Is it cost effective?
+ • Will it be accepted?
Acknowledgments

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