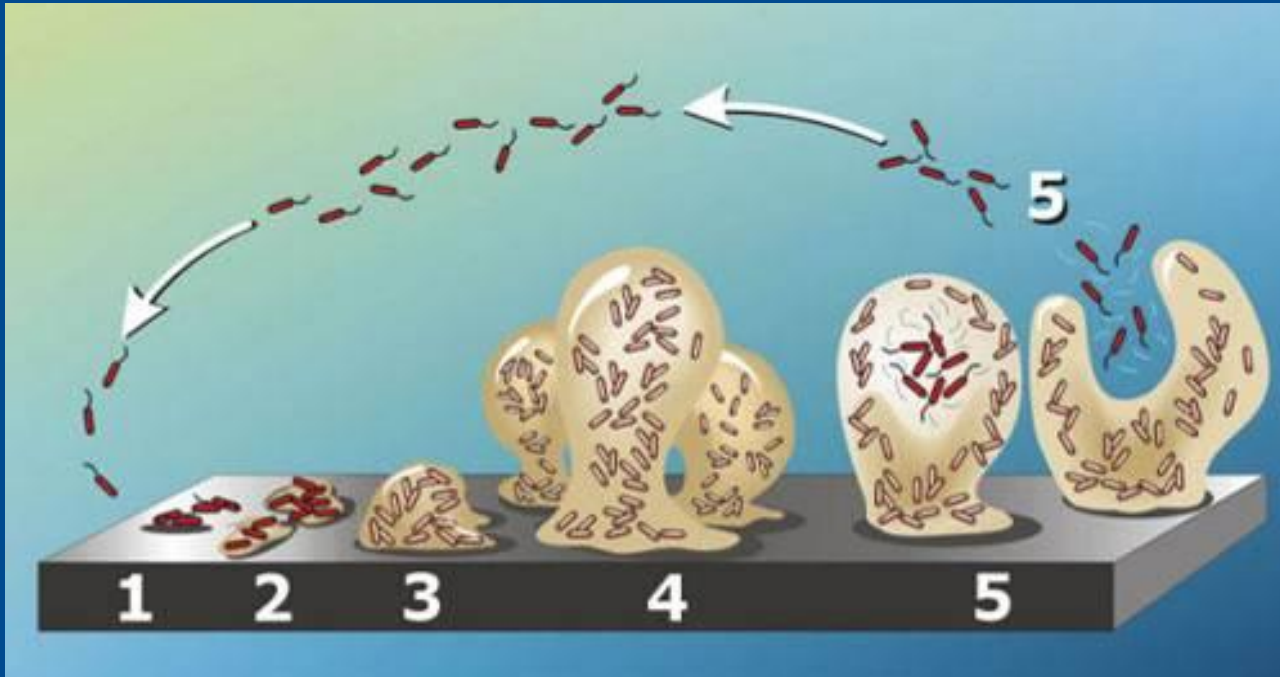


Defences

Lack of Cell Wall

- Not susceptible to the penicillins and cephalosporins that inhibit the cross-linking of amino acid chains in peptidoglycan synthesis.
- They are also resistant to sulphonamides, which inhibit folic acid synthesis, and those aminoglycosides that inhibit microbial respiration.

Defences - Biofilms



- When bacteria form a biofilm they can exacerbate disease
- Increased resistance to all stresses including the action of host defences, antibiotics and environmental stress
- Often said to be 10- 1000x more resistant compared with planktonic cells.

Biofilm

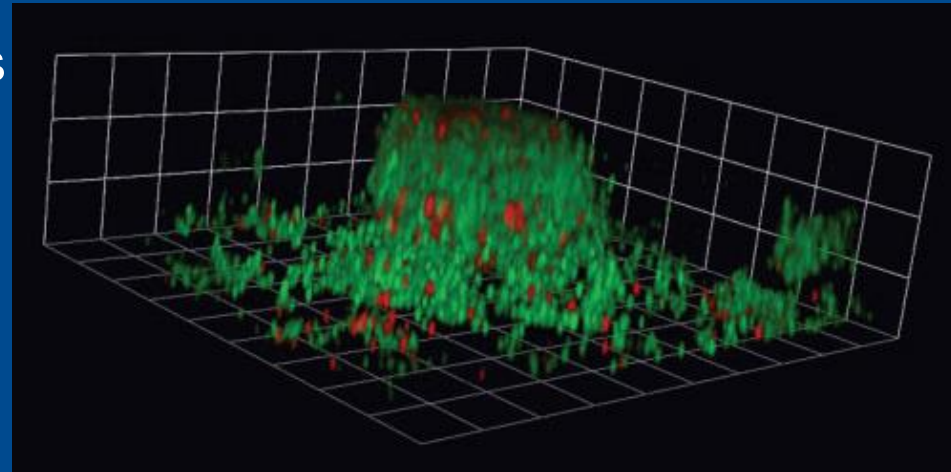
Survivability in the host, in the environment
evasion / resistance to antimicrobial treatment
resistance to disinfectant

Pathogenicity factors

Mycoplasmas lack many of the genes associated with biofilm formation in other bacteria.

Proteomic analysis and genetic analysis using defined deletion mutants.

Transcriptomics.



Biofilm – Molecular & Proteomic analysis

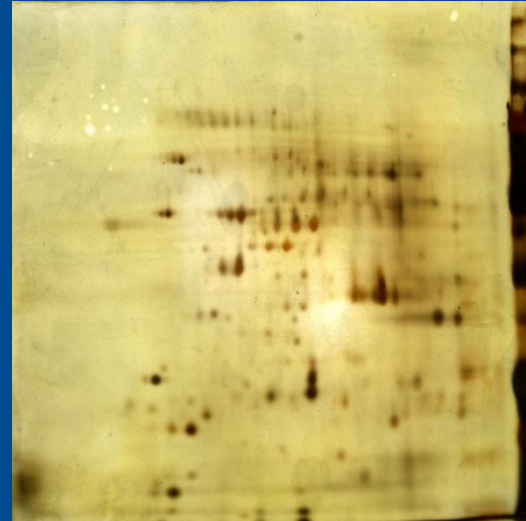
- Knockout of some genes led to dramatically decreased biofilm formation.

Defined Mycoplasma mutants donated by
J. Craig Venter Institute

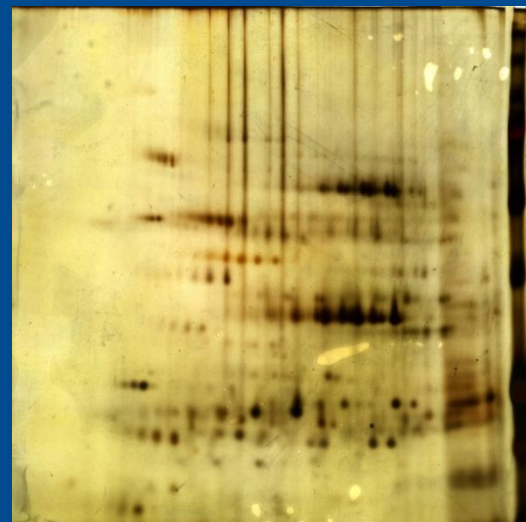
- Genes that may be essential for biofilm formation include:

- Lipoproteins
- membrane proteins
- PTS system IIBC glucose transporter
- pyruvate dehydrogenase complex

2D SDS PAGE and Mass spec analysis



Biofilm
grown cells



Planktonic
grown cells

Defences

Antigenic Variation

- Phase variation – switching ON/OFF surface antigens.
- Size variation – change in repetitive units of surface proteins.
- Variation of surface presentation – mask certain epitopes.

Variable Surface Proteins (Vsp's).

13 members of *Vsp* family - plus 36 putative variable lipoproteins

If all of the potential phase variable proteins are actually subject to high frequency switching and if all combinations are viable, there are **13 x 10²²** different variants possible!

Information from Michael Calcutt, University of Missouri, USA

Evade immune system – possible implications for vaccines

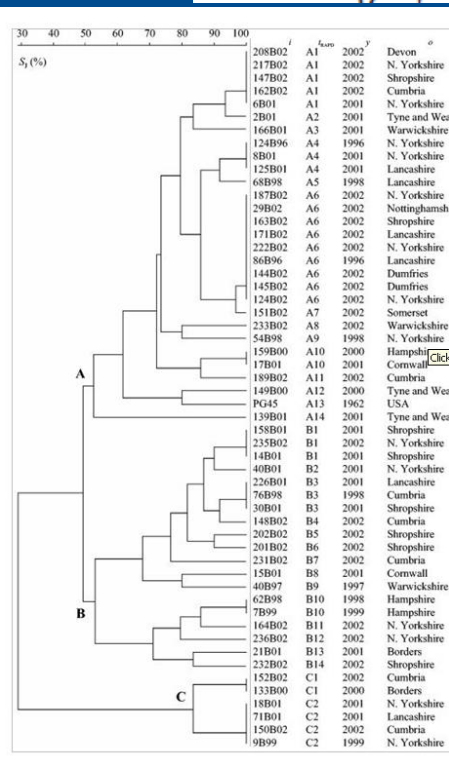
Molecular typing

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2004, p. 4556–4565
 0095-1137/04/\$08.00+0 DOI: 10.1128/JCM.42.10.4556-4565.2004
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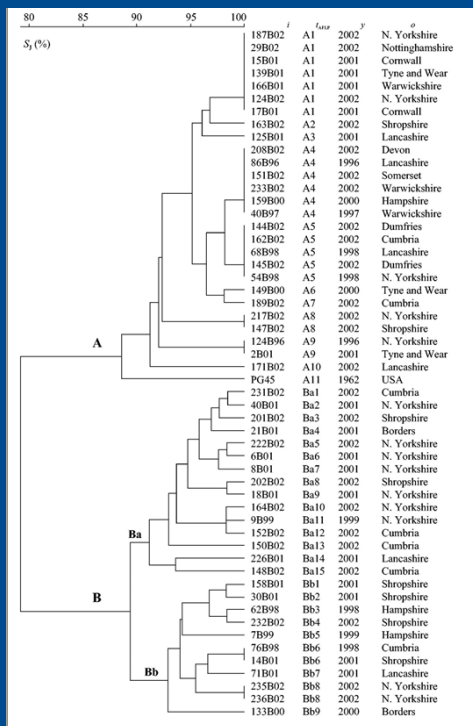
Vol. 42, No. 10

Molecular Epidemiological Analysis of *Mycoplasma bovis* Isolates from the United Kingdom Shows Two Genetically Distinct Clusters

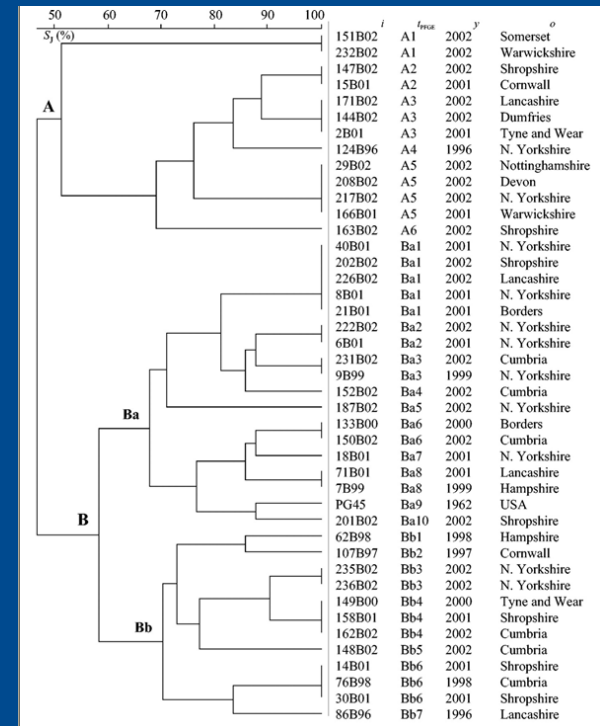
Laura McAuliffe,^{1*} Branko Kokotovic,² Roger D. Ayling,¹ and Robin A. J. Nicholas¹



RAPD



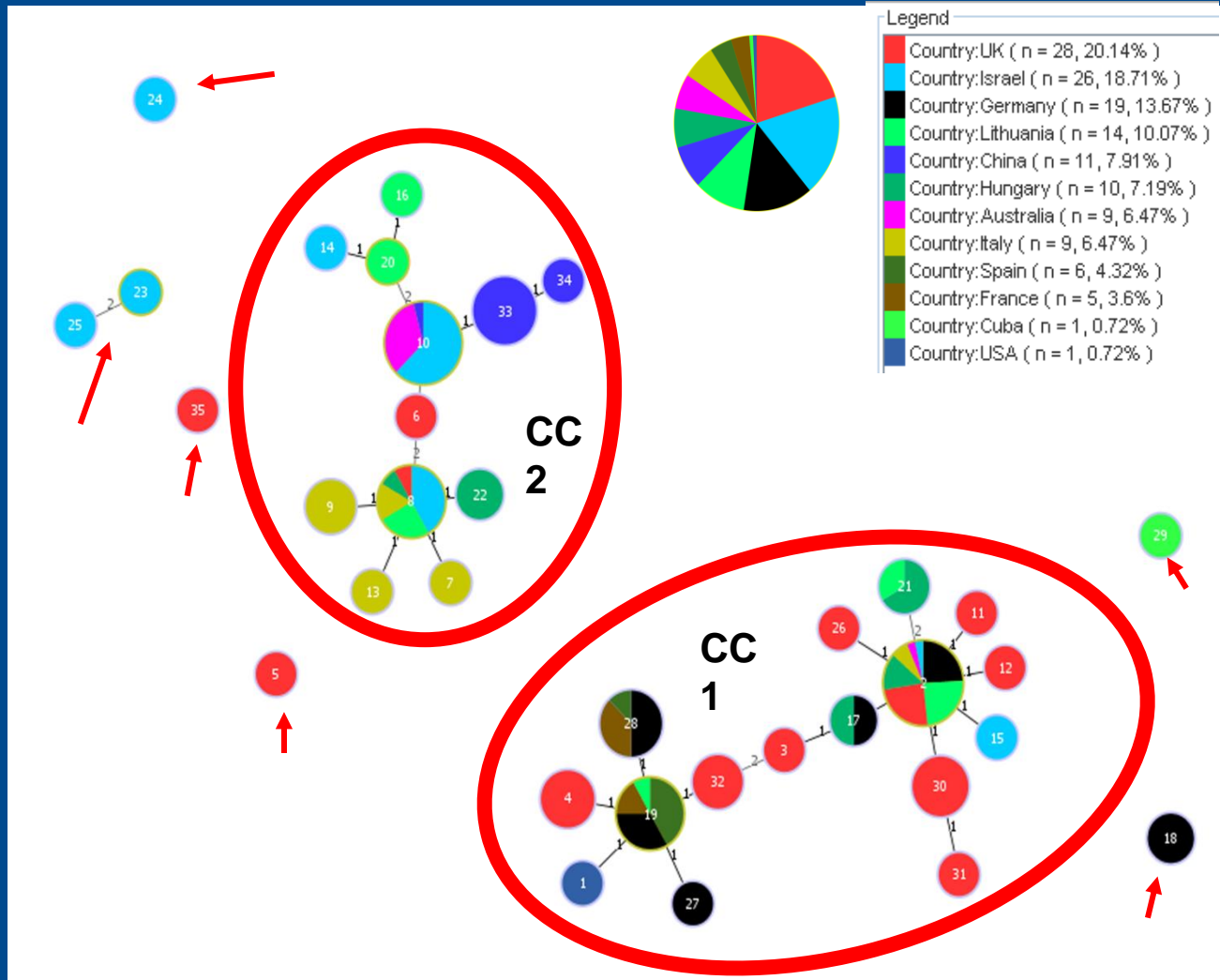
AFLP



PFGE

Molecular typing

MLST – *Mycoplasma bovis*



Control - Prevention

- Good husbandry – adequate ventilation, not overcrowding.
- Disease control measures – restricted access – change of clothes/overalls - use of disinfectant dips – regular disinfecting.
- Not introducing new animals / disease onto premises - take adequate quarantine and disease testing precautions – all in / all out practice.
- Prophylactic treatment - which one, risks such as developing resistance

Disease Prevention is better than Cure

Selection of Antimicrobials

Mycoplasma STATIC or Mycoplasma CIDAL ????

At the MIC the antimicrobial is inhibiting growth - Mycoplasmastatic (not necessary killing the organism). The organism can grow again if the antimicrobials are removed. Allows immune system to catch up.

Mycoplasmastatic – macrolides, tetracyclines, lincosamides, chloramphenicols.

Mycoplasmacidal – kills the organism – does not require help from the immune system.

Mycoplasmacidal antimicrobials are useful where infections are at sites with reduced immune system contribution – endocarditis, meningitis.

Mycoplasmacidal – aminoglycosides (streptomycin), fluoroquinolones

Selection of antimicrobials

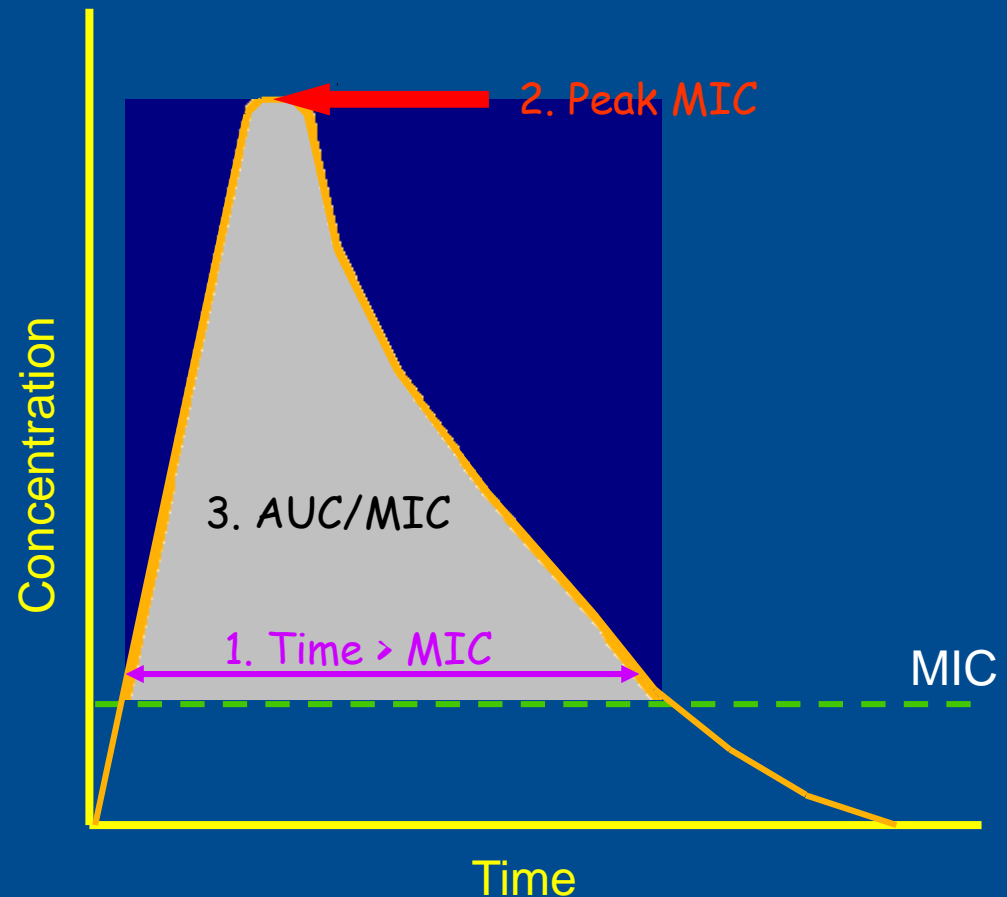
Pharmacodynamics and Pharmacokinetics

1. Time the antimicrobial remains above MIC concentration (time-dependent activity)

2. Concentration dependent activity or time-independent activity.

Ratio of peak concentration of the drug to the MIC

3. Ratio of the area under the concentration-time curve to the MIC



Example: Aminoglycosides – concentration-dependent - given as large single dose

Selection of antimicrobials

Pharmacodynamics and Pharmacokinetics

What happens to the antimicrobials in the animal?

- Inoculation - to site of action (circulating blood).
- Traverse biological membranes & fluid compartments to reach target / receptor site.
- Eliminated (circulating blood).
- Some drugs can't pass all membranes.
- Some drugs accumulate – a result of binding, dissolving in fat, or active transport mechanisms. (serum binding – important measure)
- Some drugs accumulate more in diseased organs (tilmicosin 3X more concentrate in diseased lungs than healthy lungs).
- Some drugs can enhance the immune system (macrolides enhancing phagocytosis).
- Some antimicrobials have anti-inflammatory effect and some now combined with anti-inflammatory drugs - Resflor
- Meat / Milk withdrawal times & COST.