**Acinetobacter baumannii**

*Facts and Fiction*

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**Microbiology in East London**

- Royal London Hospital
  - 1,100 bedded teaching hospital
  - Regional trauma centre
  - HEMS
- 18 bedded ITU
  - 18th century building
  - 70% of admissions from the trauma service
  - Long duration of stay

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**Rise of Antimicrobial Resistance**

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<tr>
<th>Gram positives</th>
<th>Gram negatives</th>
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<tr>
<td>Penicillins</td>
<td>1950</td>
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<tr>
<td>Tetracyclines</td>
<td>1960</td>
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<td>Aminoglycosides</td>
<td>1970</td>
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<td>Cephalosporins</td>
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<td>Quinolones</td>
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<td>Glycopeptides</td>
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<td>Linezolid</td>
<td>2000</td>
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<td>Streptogramins</td>
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<td>Daptomycin</td>
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<td>Dalbavancin</td>
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<td>Retapamulin</td>
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<tr>
<td>Tigecycline</td>
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<td>Carbapenems</td>
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**Rise in bloodstream infections due to MDR Acinetobacter in Critical Care at BLT 1998 - 2009**

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<th>Year</th>
<th>% susceptible only to polymyxin</th>
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<tr>
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<td>0.0%</td>
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**Hospital Acquired Gram-Negative Bloodstream infection at BLT (2007)**

- Acinetobacter
- Proteus
- Morganella
- E. coli
- Enterobacter
- Pseudomonas
- Klebsiella

n = 112

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**Acinetobacter baumannii**

- Gram-negative coccobacillus
- Member of family *Moraxellaceae*
  - Aerobic, non-fermentative, catalase +ve, oxidase -ve
- Natural habitat
  - Environmental? Soil? Plants?
- Opportunistic pathogen
- Very little carriage by healthy individuals
  - A. baumannii found on human skin in only 0.5%
- Infections
  - Ventilator associated pneumoniae,
  - Bacteraemia,
  - Burn wound infection
  - Prosthetic device related infection
Taxonomy of Acinetobacter

- 1980's Acinetobacter split into 12 DNA groups
  - A. baumannii, A. calcoaceticus, A. haemolyticus, A. johnsonii, A. lwoffii
- 1990's – 2000's additional species described
  - A. parvus, A. schindleri, A. ursingii from humans, many others from the environment
  - 31 species described to date 17 named
- A. calcoaceticus – A. baumannii complex
  - Closely related and phenotypically indistinguishable
  - A. calcoaceticus, Acinetobacter genomospecies 3, Acinetobacter 13TU
  - A. calcoaceticus - environmental

UK / European Epidemiology

- 2000 – A prevalent, multi-resistant clone identified in hospitals in South East England (SE Clone)
  - Majority in London
  - Intensive Care units
- 2003 – 2005 – expanded to 48 hospitals
  - OXA clones 1/2
- 3 European clones defined by PFGE
- 2009 – Carbapenem resistant A. baumannii genomospecies 3 in Ireland

Global Dissemination of A. baumannii

- Countries reporting outbreaks pre 2006 red, Post 2006 yellow

Sequence based typing Schemes

- Multi-locus sequence typing
  - 7 housekeeping genes: gltA, gyrB, gdhB, recA, cpn60, gpi, rpoD
- Multiplex PCR typing
  - cas, CmrA and OXA-23 alleles
  - Corresponds with major EU PFGE clones
- Variable number tandem repeats
  - Discrimination within PFGE types

Antimicrobial Resistance

9th Tripartite Meeting of The Celtic Microbiology Associations
(Hosted by the Welsh Microbiological Association)
Cardiff 5-7th June 2009
Antimicrobial Resistance

- Target modification
- Drug destruction
- Drug modification
- Active drug removal
- Impermeability
- Intrinsic
- Acquired
- Resistance islands
  - AbaR1 – 45 resistance genes of foreign origin
  - Class 1 integrons, transposons
- Plasmids

β-lactam Resistance

- Inherent Chromosomal AmpC (ADCs)
  - Not inducible – regulated by IS42
- Class A Extended-spectrum β-lactamases
  - VEB 1 – clonal dissemination in France
  - PER 12 – pleomorphic chromosomal – IS552 regulated
- TEM-52/SHV-12, CTX-M/43
- Carbapenemases
  - Class A – not yet detected
  - Class B metallo-β-lactamases
    - VEB-1
    - clonal dissemination in France
    - PER 1/2 – plasmid / chromosomal
    - IS552 regulated
  - TEM-92/116, SHV-12, CTX-M/2

- Ciprofloxacinases
  - aac(6’)-Ib-cr

- Outer membrane protein loss
  - CarO porin

Efflux Pumps

- Major facilitator Superfamily (MFS)
  - TetA – tetracycline
  - TetB – tetracycline, minocycline
  - CmlA – chloramphenicol
  - AdeABC – β-lactams, aminoglycosides, macrolides, Chloramphenicol
  - AdeIJK – Above + fusidic acid, trimethoprim
  - MATE family
    - AdeM
      - quinolones, gentamicin, trimethoprim

Aminoglycosides

- Inactivation of aminoglycosides by
  - Phosphorylation – APH enzymes
  - Acetylation – AAC enzymes
  - Adenylation – ANT enzymes
- Pan-resistance to aminoglycosides
- 16S rDNA methyltransferases
- ArmA – plasmid mediated / transposon
- Efflux
  - AbeM (MATE family pump)

Quinolone Resistance

- DNA gyrase mutations
  - GyrA, GyrB, ParC, ParE – high level resistance
- Efflux
  - Most quinolones are substrates for AdeABC and AdeM
- Ciprofloxacinases
  - aac(6’)-Ib-cr – variant

Mechanisms of MDR in A. baumannii OXA-23 clone 1

- β-lactams
  - TEM-1 penicillase
  - Overexpression of chromosomal AmpC
  - OXA-51 and OXA-23 carbapenemase
- Aminoglycosides
  - aac(3)-Ia, aac(3)-Ib, aac(6’)-Ib-cr
  - armA – IS552 16S rRNA methyltransferase
- Quinolones
  - OQRDR GyrA/B, ParC/E mutations, AdeABC efflux
- Trim / Sul
  - Aaf
- Tetracyclines
  - tetL
  - adeABC – multidrug efflux pump
- Polymyxin
  - Difficult to detect
  - Heteroresistance described

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Clinical Manifestations / Associations

- Hospital Acquired Pneumonia
  - Commonest site of isolation is respiratory tract colonisation v infection?
  - US study – 5-10% of all ICU HAP v VAP
- Community Acquired Pneumonia
  - Fulminant pneumonia with bacteraemia and septic shock
  - Alchoholic in Tropical Australia and SE Asia
  - Reality A. baumannii – identification problems?
- Bloodstream Infection
  - Usually ICU associated, mean time of onset 26 days post admission
  - 2ndary to lines, pneumonia, UTI or wounds
- Wound Infections
  - ICU acquired SSTI and burn wound infections
  - Commonest isolate from traumatic military injuries – Iraq / Afghanistan
- Neurosurgical meningitis
  - EVD associated ventriculitis

Pathogenesis of A. baumannii infection

- Comparative genomics A. baumannii v A. baylyi
  - Type IV secretion system, pilus biogenesis, Fe uptake and metabolism
- Siderophores
  - Expression highly strain dependent
  - Antibodies in sera of patients with A. baumannii bacteraemia
- Biofilm formation
  - Readily adheres to inanimate objects
  - Pilus biogenesis involving cas operon

Pathogenesis

Stages in Microbial Pathogenesis

- Attachment and Colonisation
- Exit the Host
- Evasion of Host Defences
- Dissemination and Systemic Disease
- Complicated interaction between host and organism derived factors

Interactions with Respiratory Epithelial Cells

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<th>MOI</th>
<th>Time (h)</th>
<th>OXA 23-1</th>
<th>MOI 1</th>
<th>MOI 10</th>
<th>Type strain</th>
<th>MOI 1</th>
<th>MOI 10</th>
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**A. baumannii Lipopolysaccharide**

- A. baumannii LPS is a potent activator of TLR-4
- Stimulates both TLR-4 AND TLR-2 on human cells
- Antibodies to A. baumannii are widespread in healthy individuals
- Erridge, 2007
- Morgan and Porton, 2009

**Metabolic Diversity of Acinetobacter**

- Acinetobacter loves sugar!
- Acinetobacter loves alcohol!
- Acinetobacter loves fat!

**Acinetobacter and Sugar?**

- Acinetobacter burn wound infection associated with impaired glucose tolerance
  - 9.8 X increase in impaired glucose tolerance p< 0.0001
  - Independent of diabetes status
  - Independent of burn severity
- Why?
  - Acinetobacter metalloprotease
  - Cleaves insulin, IGF-1 and glucagon
- How?
  - No insulin / hormone levels measured
  - Enzyme is periplasmic — not secreted

**Acinetobacter and Alcohol?**

- Acinetobacter can grow in the presence of small quantities of ethyl alcohol
- Exposure to alcohol makes A. baumannii pathogenic to C. elegans

**Growth of MDRAB in Ethanol Supplemented Media**

- Growth of OXA 23 clone-1
- Growth of OXA 23 clone-2
- Growth of South East Clone

**Growth of MDRAB in Media Supplemented with Alcohol Based Handrubs**

- Growth of MM Only
- Growth of 1% Softalind
- Growth of 1% Skinman
- Growth of 1% Purell
- Growth of 1% Spirigel
Acinetobacter and Fat:
- Lipase production well characterised in non-baumannii species e.g. A. veronii
- Lack of subcutaneous fat in burn wound grafts
- MDRAB produce lipases
- Which are enhanced by some antibiotics...

Clinical Impact, Treatment and Control

Studies on the Clinical Impact of A. baumannii
- Dozens of studies but little consensus!
- Enormous methodological heterogeneity
- Prospective v retrospective
- Case control v cohort
  - What defines a ‘case’ – polymicrobial infections / different sites etc
  - What defines a ‘control’ – no A. baumannii infection, infection with something else, colonisation v infection
- Appropriate ‘Matching’ for co-morbidities
- Problems with speciation
- Geographical variation
  - Impact of individual clones
- Most studies identify excess hospital / ITU stay
- No consensus on attributable mortality!

Does A. baumannii Infection have Attributable Mortality?
- Yes
  - Lortholary, 1995 – infection / colonisation v not
  - Garcia-Garmendia, 1999 – infection / colonisation v not
  - Abbo, 2007 – isolation of MDRAB v not
  - Grupper, 2007 – A. baumannii bacteraemia v no bacteraemia
  - Kwon, 2007 – Imipenem S v Imipenem R infection
- No
  - Blot, 2003 – A. baumannii bacteraemia v no bacteraemia
  - Garnacho, 2003 – A. baumannii VAP v no A. baumannii infection
  - Albrecht, 2006 – A. baumannii infection v colonisation in burns
  - Loh 2006, A. baumannii in respiratory secretions v not
  - Sunenshine, 2007 CDC – MDRAB v no infection

Mortality from MDR A. baumannii Bacteraemia at BLT:
Retrospective cohort study of 369 Acinetobacter spp bacteraemia
(MDRAB n=78)
Impact of carbapenem R, polymyxin Rx and ITU care

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Impact of ITU Requirement on Mortality from MDRAB Bacteraemia

A. baumannii bacteraemia is a surrogate marker of mortality in ITU?

‘Polymyxin E and Polymyxin B - Colistin’

- Colistin susceptibility testing
  - Disc testing is unreliable
  - Bactericidal in vitro but ‘heteroresistance’ with regrowth described in time kill assays
- Polymyxin formulations
  - Colistimethate sodium a prodrug metabolised to colistin
    - Parenteral and nebulised formulations
- Colistin sulphate
  - Oral and topical use
- Uncertain pharmacokinetics
  - Commercial preparations contain different amounts of active drug
  - Never subjected to formal drug development programme
  - If colistin were a new drug in 2009 would it receive a licence?
  - Concerns over nephrotoxicity and neurotoxicity not seen with modern use

Unorthodox Treatment Regimens v MDR strains

- In-vitro studies of colistin containing combinations
  - Polymyxin B + imipenem + rifampicin – Synergy
  - Colistin + rifampicin – Synergy
  - Colistin + meropenem + azithromycin – Synergy
- In-vivo studies of colistin containing combinations
  - Colistin + rifampicin – effective in mouse pneumonia and rat model
  - Case reports
    - Colistin + rifampicin – ‘favourable response’
  - Sulbactam
    - Intrinsic activity via PBP 2 inhibition
    - Option in isolates susceptible in vitro?
    - Some reports of clinical efficacy in non-severe infections

Tigecycline?

- Brought to market as a Gram-positive agent for skin and soft tissue infections
- Good in-vitro activity v MDRAB
- Bacteriostatic
- Rapid emergence of resistance due to overexpression of efflux pumps
- Case series of infections involving MDRAB treated with tigecycline
  - 68 % clinical response rate
  - 41 % overall mortality
  - Recurrent episodes of bacteraemia with development of frank resistance in 3 cases
- Very low serum levels 0.8 mg/L
- Sub-MIC for most strains of A. baumannii (1.5 mg/L)
- BSAC breakpoints S< 1 R >2

Gordon, JAC 2009

Infection Control Considerations

- Environmental decontamination paramount
- A. baumannii very resilient to desiccation
- Antimicrobial policies
  - Most antibiotics implicated as risk factors for acquisition and persistence
  - Increased reliance on carbapenems probably implicated in dissemination of OXA-clones
- Isolation
  - Successful in some instances, may be impractical
  - Screening / decontamination
    - Patients often have enteric carriage – selective CHROMagar
    - Enteral polymyxins for SDD?
    - Nebulised polymyxins for respiratory colonisation?
    - Need RCT data

A. baumannii: Facts and Fiction

- Facts
  - Successful clones of A. baumannii disseminated worldwide
  - The organism has a formidable capacity for capturing antimicrobial resistance genes
  - It has become exceptionally adapted to the hospital
- Fiction?
  - The pathogenesis of A. baumannii infection is well understood
  - A. baumannii infection has major clinical impact
  - Polymyxins and tigecycline are effective treatments for sensitive strains
  - Is it the Gram-negative MRSA – or is it the Gram-negative CoNS?
Increase in Pubmed indexed articles on *A. baumannii* 1997-08

### Acknowledgements

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<th>Microbiology SpRs</th>
<th>Consultant Colleagues</th>
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<td>Nicola Gordon</td>
<td>Michael Millar</td>
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<td>Rhys Khan</td>
<td>Daniel Krahe</td>
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<td>Judi Edwards</td>
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**Queen Mary University**

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