Antibiotic resistance
a mechanistic overview
Neil Woodford

Antibiotic Resistance
a Mechanistic Overview

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Consultant Clinical Scientist

Mechanisms of antibiotic action

Resistance is as old as antibiotics
(not just human use of them)

- Penicillin isolated in 1928
- Resistant E. coli ‘discovered’ in 1940
- …but antibiotics and bacteria have co-existed for millions of years
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Antibiotic resistance mechanisms

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Types of resistance

- Intrinsic (or inherent) resistance
  - Resistance to an agent is normal for a genus, species or bacterial group (lack the target, or drug can’t get to target)
    - Glycopeptide resistance in Gram-negatives
    - Aztreonam resistance in Gram-positives
- Acquired resistance
  - Most isolates of a genus, species or bacterial group are susceptible, but resistance may arise via:
    - Mutation (usually of a chromosomal gene) e.g., Rif R, FQ R
    - Acquisition of new DNA conferring resistance (horizontal spread)

Defining resistance

- Biological - “the inhibition zone is smaller (or MIC is higher) than normal for the species, so it’s resistant”
- Pharmacological - “the MIC is 32 mg/L, but the drug has a serum peak of 150 mg/L, so it’s sensitive”
- Clinical - “I know that strains like this don’t respond in the patient”
Susceptibility / resistance of every bacterial isolate reflects interplay of multiple factors

### Antibiotics select resistant bacteria: mutational resistance

- A mutant emerges randomly
- Sensitive bacteria killed by antibiotic
- Mutant's progeny survive and grow

I have called this principle, by which each slight variation, if useful, is preserved, "Natural Selection"

### Bacteria carry resistance in their DNA

- Mutations in chromosomal DNA can cause resistance
- Many bacteria have extra DNA in small rings, known as plasmids
  - Plasmids can also carry resistance
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...and they don’t keep resistance
to themselves

Three routes that bacteria use
to transfer resistance

- Most efficient
  between closely related bacteria
- ..., but unrelated bacteria often exchange DNA too

http://bioinfo.bact.wisc.edu/themicrobialworld/homepage

The Red Queen Principle

- Evolution is often an “arms race”
- Antibiotic development vs. antibiotic resistance
- Bacteria evolve in “real-time”

“Now, here, you see, it takes all the running you can do, to keep in the same place”


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Resistance is inevitable…
even to new antibiotics

- Considered by all companies developing new agents
- Search for cross-resistance
  - Assess activity vs. clinical strains resistant to other agents
  - ? resistance reservoir if product is natural (or semi-synthetic)
- In-vitro development of resistance by mutation
- Not reliable predictors; Ideal scenarios:
  - Penicillin vs. Strep. pyogenes: no resistance
  - Vancomycin: resistance emerged after c. 30 years use
- Will resistance to compound ‘X’ emerge:
  - Quickly, in target species, … and will it be transferable?

How quickly does resistance emerge?

- Linezolid: a synthetic drug
  - Bacteria have never ‘seen’ anything like it
  - Excellent activity against almost all Gram-positive species
  - Clinical use sets them a new challenge

Oxazolidinone timeline

Influenced by many factors, including:
- Use of agent (how much, by whom?)
- Cross-resistance to other antibiotics
- Type of resistance

<table>
<thead>
<tr>
<th>Class discovered</th>
<th>Investigation restarted</th>
<th>UK licensing of linezolid</th>
<th>1st LRE in UK</th>
<th>Will we have a major resistance problem?</th>
</tr>
</thead>
</table>

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Mechanism 1: alteration of the target
Linezolid resistance and 23S rRNA

Only G2576U
in clinical isolates
(MICs, 8-128 mg/L)

Prystowsky et al., AAC 2001;45:2145-56

The forensics of antibiotic resistance

➢ Resistance involves
  • Emergence of mutations
  • Spread of resistance genes
    (plasmids, transposons, integrons)
  • Spread of resistant strains and clones of bacteria

➢ Tracking and characterizing
  • The resistant strains: in hospitals and in the community
  • Their resistance genes
  • Surveillance and good microbiology

Epidemiological investigation
can be applied to every level

Host species
Strains, clones, phylogenetic
groups, virulence traits, co-resistance

Patients
Hospital / community setting; risk factors

Genes
Gene carriers
IS, In, Ty, plasmids

Courtesy of Rafael Canton

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**Surveillance of resistance**

- Informs on prevalence and changes in antibiotic resistance
  - Guides empirical prescribing & control strategies
  - Assess if control is working
- Surveillance shortfalls
  - Lack of clinical denominators
  - Need more community based surveillance
  - Need to link antibiotic consumption to resistance
- Must be supported by good microbiology (not just number crunching)

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**Similar trend, but is there a causal association?**

<table>
<thead>
<tr>
<th>Year</th>
<th>% MRSA bacteraemias</th>
<th>Rise of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>'93</td>
<td>1</td>
<td>E-15 (ST33)</td>
</tr>
<tr>
<td>'94</td>
<td>2</td>
<td>E-16 (ST34)</td>
</tr>
<tr>
<td>'95</td>
<td>3</td>
<td>E-3 (ST05)</td>
</tr>
<tr>
<td>'96</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>'97</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Data: HPA

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**Mechanism 2: metabolic by-pass**

β-lactam resistance in MRSA

- Methicillin inhibits PBP1, 2, 3
- New peptidoglycan
- Cross-linked wall
- MRSA produce PBP2', decreased binding, clinical resistance to most available β-lactams
- Ceftobiprole
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**Cephalosporin-resistant E. coli from bacteraemias**

- An explosive increase recorded since start of 21st century
- In the UK:
  - c. 20,000 cases E. coli bacteraemia p.a. (voluntary)
  - c. 12% CTX and/or CAZ resistance = c. 2400 cases p.a.

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[Map showing cephalosporin-resistant E. coli from bacteraemias]

http://www.earss.nivm.nl

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..., but hang on a minute; Why might resistance rates rise?

- Technical (artefacts)
  - Change in surveillance methods (e.g., mandatory vs. voluntary)
  - Lowering of breakpoints (isolates previously S, now R)
  - Education / awareness (more people look, and so find)
  - Better screening methods
- Biological (real)
  - Expansion of resistant clones / strains
  - Emergence of resistance in new clones / strains
    - De novo emergence (mutation)
    - Horizontal spread of plasmids between strains

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**Understanding rising prevalence:**

SE England, 2004

![Bar chart showing relative prevalence of E. coli, K. pneumoniae, and Enterobacter spp. resistant to various antibiotics](chart.png)

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Mechanism 3: drug destruction
β-lactamases:
- Oxyimino-aminothiazolyl or methoxy groups:
  - Evade classical penicillinas
  - Hinders access to active site
  - No hydrolysis
- ESBLs are able to hydrolyse β-lactam bond
  - Allow hydrolysis
  - Confer resistance

Global explosion of CTX-M ESBLs in Enterobacteriaceae

Multi-resistance plasmids: how bad is bad?

<table>
<thead>
<tr>
<th>Antibiotic classes</th>
<th>Genes</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>aadE'-Ib-cr</td>
<td>Modify drug</td>
</tr>
<tr>
<td></td>
<td>aadA5</td>
<td></td>
</tr>
<tr>
<td>β-lactams</td>
<td>blaCTX-M-15</td>
<td>Destroy drug</td>
</tr>
<tr>
<td></td>
<td>blaoxa-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaoxa-5</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>catB4</td>
<td>Modify drug</td>
</tr>
<tr>
<td>Macrolides</td>
<td>mph(A)</td>
<td>Efflux</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>aadE'-Ib-cr</td>
<td>Modify drug</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>sulI</td>
<td>By-pass</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>dhfr1</td>
<td>By-pass</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>tetA</td>
<td>Efflux</td>
</tr>
</tbody>
</table>

pEK499 (118 kb) encodes CTX-M-15 ESBL in a prevalent UK strain of E. coli
Woodford, Carattoli et al., AAC

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**E. coli & Klebsiella with ESBLs or AmpC: can it get worse?**
- Carbapenems
  - Standard i.v. therapy for ESBL / AmpC producers

...which leads to an all-too-familiar situation
- ↑ use = ↑ selective pressure = ↑ resistant isolates

**Mechanism 4: reduced uptake porin-mediated carbapenem resistance**
- *E. coli* strain A’ “usually” encodes 3 β-lactamases:
  - CTX-M-15; OXA-1; TEM-1
- In 1 centre it has acquired an additional AmpC β-lactamase:
  - CMY-23
- ...and carbapenem resistance
  - isolate A2: ETP, 4 mg/L, IPM / MEM, 0.5-1 mg/L

**Restore porins and reverse carbapenem resistance**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ETP</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>pTR</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>pTRompK36</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Doumith et al., JAC 2009; 63: 659-67

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Carbapenemase-mediated resistance in the UK

Carbapenemase producers: ARMRL referrals

Another multi-resistant epidemic bacterial clone: A. baumannii, OXA-23 clone 1

- Prevalent UK strain
- First appeared in 2002
- >50 UK centres affected
- Multi-resistant lineage
- Typically susceptible only to COL and TIG

Mechanism 5: up-regulated efflux
Tigecycline resistance in A. baumannii:

TIG MIC: 0.5 16 64
Recap: mechanisms of resistance

- Target site modification / protection (mutation or enzymic)
  - e.g., changes in a PBP, the ribosome, DNA gyrase
- By-pass (acquired target unaffected by antibiotic)
  - e.g., PBP2', mupirocin R resistance; trimethoprim resistance
- Enzymic inactivation / modification of antibiotic
  - e.g., β-lactamases, aminoglycoside-modifying enzymes
- Impermeability (porin loss)
  - e.g., ETP R Enterobacteriaceae; OprD (D2) in Ps. aeruginosa
- Active efflux
  - e.g., tigecycline resistance in A. baumannii; non-specific (affects multiple drug classes); diverse pump types
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Summary
- Resistance is complex
  - Not a new phenomenon; ‘discovered’ by us in last 70 years
  - New drug = new selective pressure = bacterial response; mechanisms are diverse
- ESBLs (CTX-M types) in E. coli are a major new resistance problem for the 21st century
  - Resistance associated with plasmids encoding multi-resistance
  - Potential to develop further resistance; mutation and other plasmids
- Surveillance & microbiology to understand dominant & emerging resistances
- Rational antibiotic usage needed to limit increasing resistance

Acknowledgements
- Colleagues at the Health Protection Agency
- Students and collaborators, 1988-2009
- Thank you for listening