

Infection Control

Shutting the gate after the horse has bolted.

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Multi-resistant bacteria (MRO's)

- ⌘ Excess mortality.
- ⌘ Excess costs.

What are we doing about it?

- ⌘ Monitoring of patients endogenous flora/environmental sampling.
- ⌘ MRA & MRSA (VRE).

Conditional phenotypes

- ⌘ not detected by phenotypic methods.
- ⌘ carbapenemases lead to variable resistance e.g. *Oxa23* and *Imp4* in enterics.
- ⌘ potential for transfer.

What use is made of it

- ⌘ 128 Intensivists ; 108 ID/ Micro
- ⌘ 90% ID / 74% ICU agree:
- ⌘ R is a clinical problem that needs action.
 - ☒ only 39% ICU used local lab. databases
 - ☒ only 68% ID used local lab. databases

(Sintchenko *et al.*,
2001)

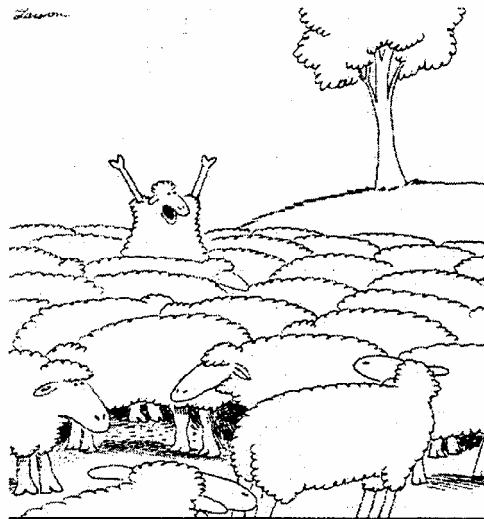
ICU – the Reality

- ⌘ highest ratio of nosocomial infections.
- ⌘ amplification of MRO by broad-spectrum antibiotic usage.
- ⌘ large ill-contained poly-microbial populations.

Where to from here?

Phenotypic vs Molecular?

- ⌘ phenotypic not working – the horse has already bolted.
- ⌘ selected MROs only.
- ⌘ no monitoring of transferable elements.



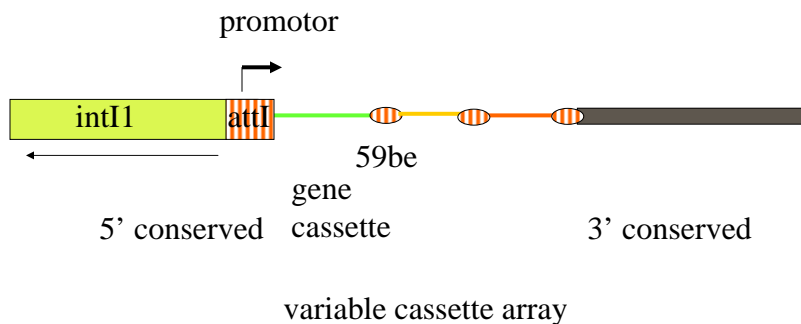
Wait! Wait! Listen to me...We don't have to be just sheep!

ICU STUDY 2004 - New tools, old problems

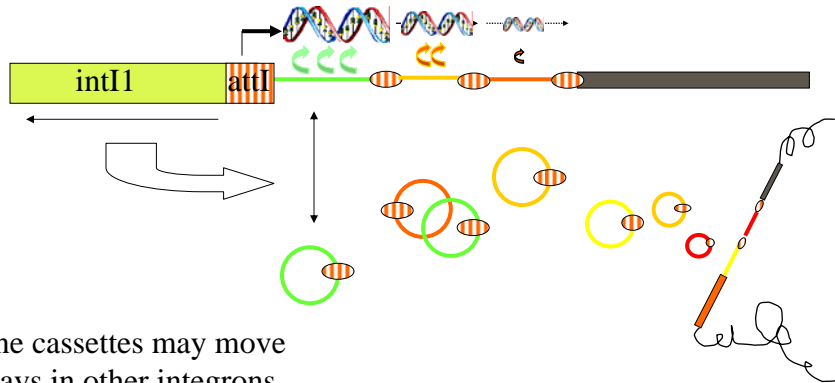
Molecular surveys

- ⌘ integrons - gene capture systems.
- ⌘ plasmids - gene transfer systems.
- ⌘ screen for specific resistance genes
eg *oxa23*. *imp4*.

what are integrons?



...a class I integron



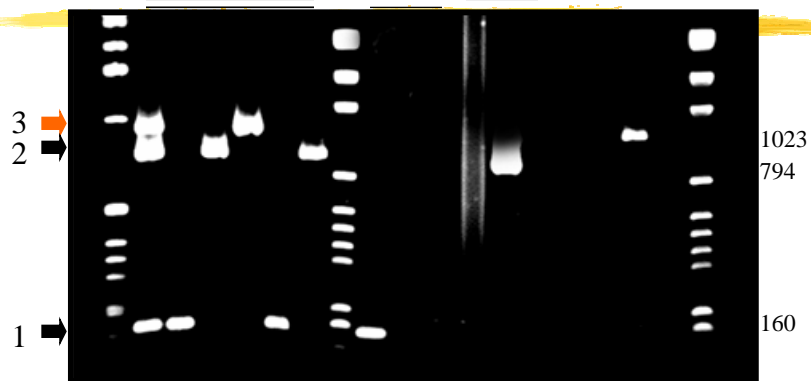
1. gene cassettes may move to arrays in other integrons
2. silent genes at end of array can be moved to the front

Collis et al., 1993. Mol Microbiol 9:41-52

multiplex

uniplex

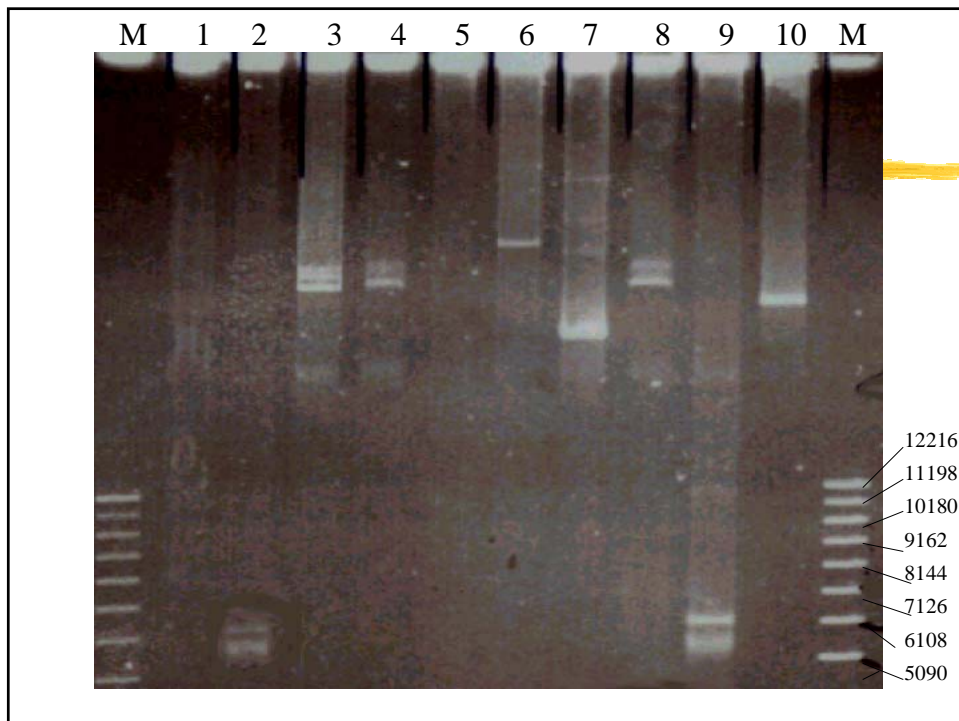
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 (b)



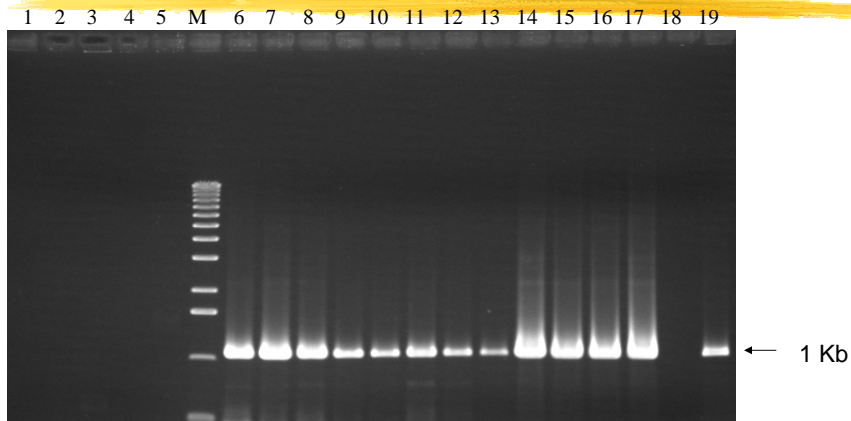
- 1: class 1,2,3 templates (S Partridge, R Hall)
- 2: class 1 template
- 3: class 2 template
- 4: class 3 template
- 5: lysate *Acb* class 1
- 6: lysate *Kpn* class 2 (M Leroi)

Plasmids

- ⌘ acquisition of plasmids.
- ⌘ transfer of resistance.
- ⌘ methods of detection/typing.



PCR screen for specific resistance genes - *oxa 23*



Lane 1 - 5 Imipenem sensitive strains of *A. baumannii*; : 1Kb molecular weight marker; lane 6 - 17 Imipenem resistant strains of *A. baumannii*; lane 18: Negative control *DH5α E. Coli*; lane 19: Positive control: *Klebsiella pneumoniae* control containing OXA-27 like gene.

Study in Haematology ward

⌘ CI 38 – *Citrobacter freundii*

⌘ CI 40 – *Klebsiella pneumoniae*

⌘ both in haematology unit, Feb 2002.

⌘ presented with positive blood cultures
12 days apart.

Study in Haematology ward

- ⌘ treatment failure whilst on Timentin.
- ⌘ identical gene cassette array.
- ⌘ would not have been found by phenotype.

Inter-patient transfer?

day isolated	gene isolated	patient id
day 50	<i>dhfr12,orfX,aadA2</i>	perineal sample patient 12
day 67	<i>dhfr12,orfX,aadA2</i>	B/C isolate pt 38. <i>C. freundii</i>
day 75	<i>dhfr12,orfX,aadA2</i>	perineal sample patient 31
day 79	<i>dhfr12,orfX,aadA2</i>	B/C isolate pt 40. <i>K pneumoniae</i>

mobile β -lactamase (*oxa1*) gene

- ⌘ E coli with *oxa1* gene isolated from blood culture.
- ⌘ mobile – on transconjugant plasmid.
- ⌘ matches integron in Salmonella plasmid.
- ⌘ altered ribosomal binding site.

Study Results

- ⌘ infection control relevance demonstrated.
 - ⊠ transconjugant plasmid: *oxa1* (?improved RBS).
 - ⊠ *Citrobacter* and *Klebsiella* with identical arrays.
- ⌘ integrons are a marker for a mobile element (vehicle) eg. plasmids, transposons.
- ⌘ integrons did not always encode the relevant phenotype.

ICU STUDY 2004

- ⌘ evaluate micro-flora and transferable resistance.
- ⌘ integron surveys – conditional phenotypes.
- ⌘ specific resistance markers.
- ⌘ plasmid surveys.

ICU STUDY 2004

- ⌘ gram negatives.
 - GI/ perineum
 - airways (ETT)
- ⌘ clinically significant isolates.

Shutting the gate - the practicalities

- ⌘ transport & storage.
- ⌘ timely specimens.
- ⌘ clinical support/Data collection.

Transport & storage

100 ul subculture from	Growth on HBA	Conditions prior to subculture
1:100,000 diln in saline	5 colonies	No incubation
Enrichment broth	650 colonies	Overnight enrichment
Enrichment broth	1060 colonies	96 hrs 4°C then overnight enrichment

Overnight enrichment/storage

Patient	Multiplex from lysate direct	Multiplex x lysate (post enrichment & -70)
1	Neg	Pos
2	Pos	Pos
3	Neg	Pos
4	Pos	Pos
5	Pos	Pos
6	Pos	Pos
7	Pos	Pos
8	Pos	Pos

Shutting the gate

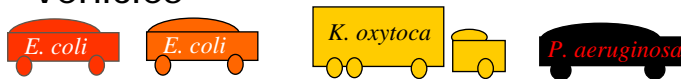
- ⌘ relevance of "old" genes (aadA; dhfr)
- ⌘ cost-effectiveness of screening
 - ☒ lysates PCR vs culture, ID, sens.
- ⌘ how do niches differ (ETT vs perineal).
- ⌘ how does antibiotic selection influence this.
- ⌘ Predictive value.

...a proposed way forward

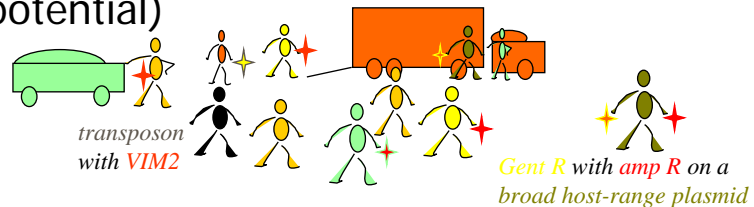
- ⌘ new technologies.
- ⌘ reliable targets.
- ⌘ cost effective.


...a proposed way forward

- ⌘ 1. establish a profile of circulating vehicles



- ⌘ 2. elements within (mobility/ range/ potential)





The world we have made as a result of the level of thinking we have done thus far, creates problems we cannot solve with the same level of thinking at which we created them.

Albert Einstein

Acknowledgements



- ⌘ Jon Iredell
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