

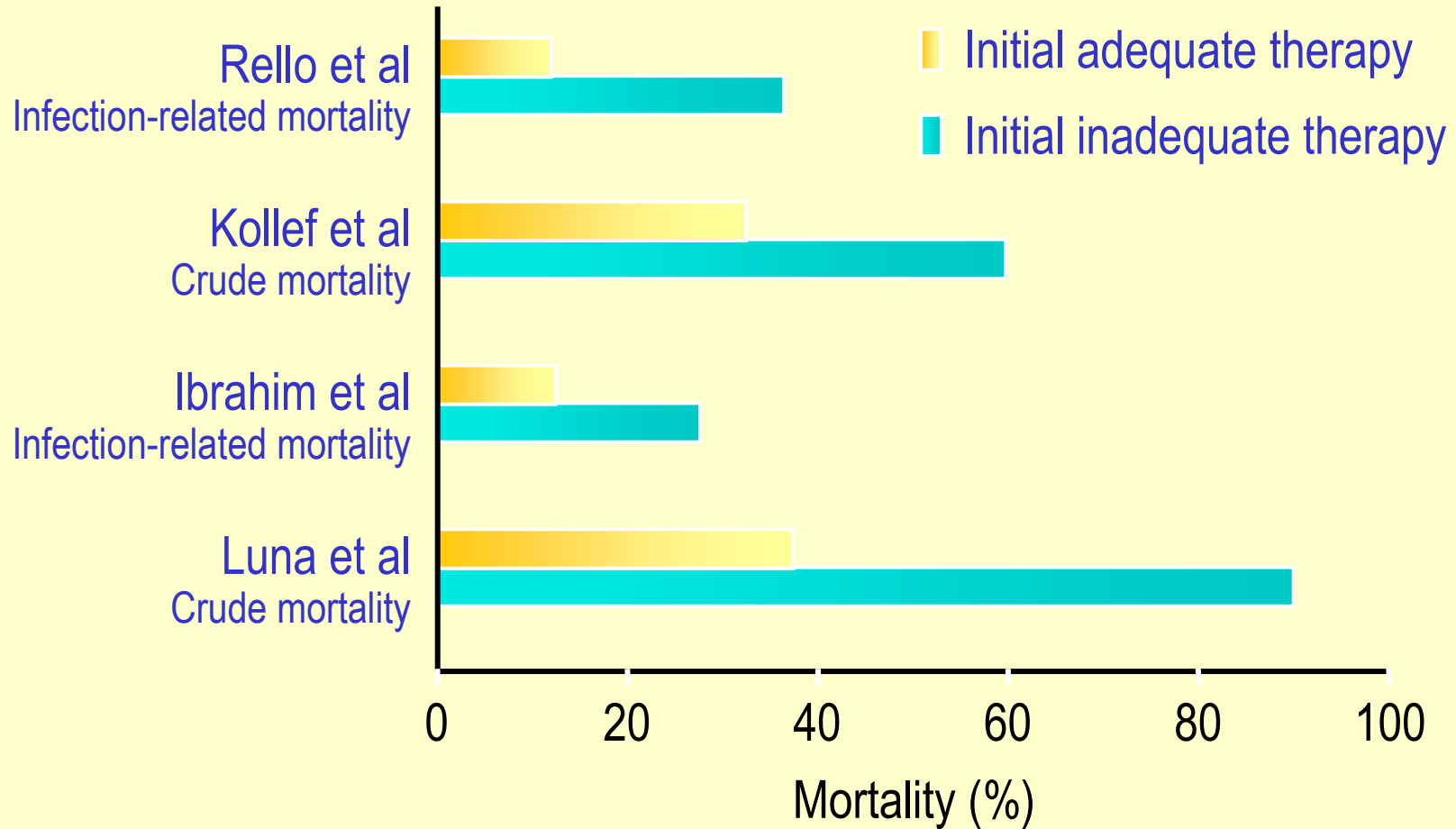
# **“real-time” identification and surveillance of antimicrobial resistance**

CIDM-PH  
diagnostics workshop 29/2-1/3/08

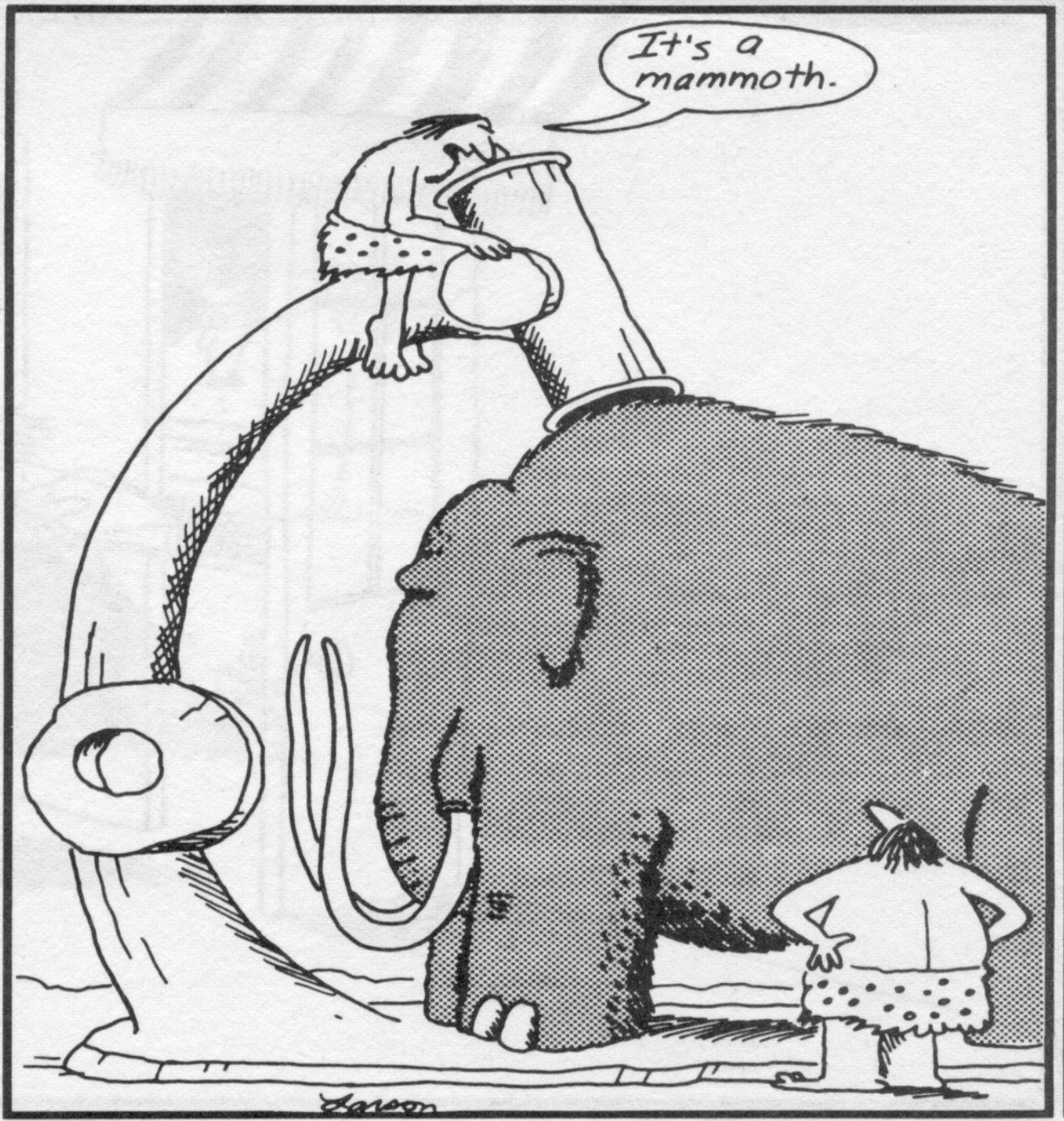
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# some results can't wait



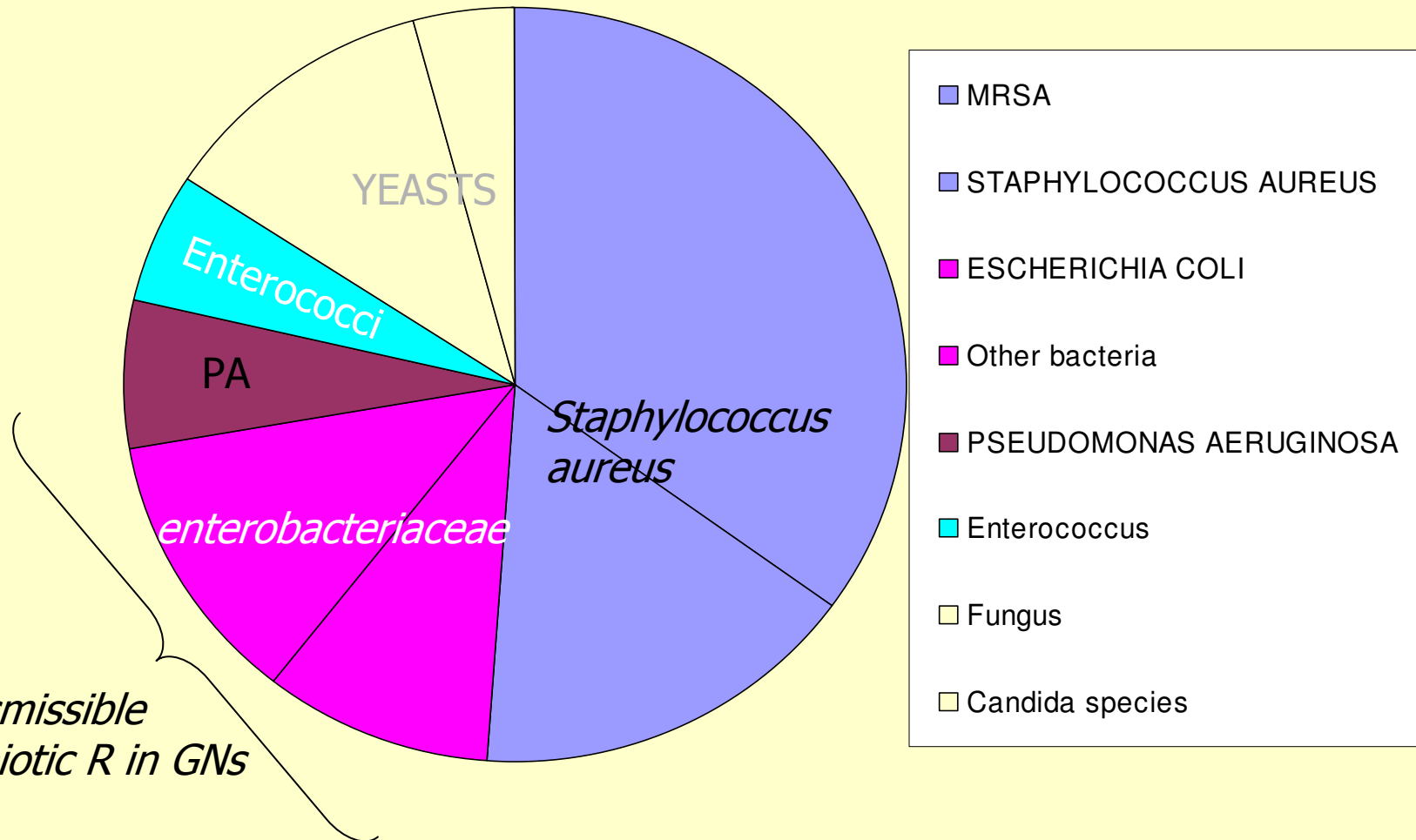
*Rello et al. Am J Respir Crit Care Med 1997;156:196–200; Kollef et al. Chest 1998;113:412–420  
Ibrahim et al. Chest 2000;118:146–155; Luna et al. Chest 1997;111:676–685*



It's a mammoth.

Larson

12% Australian ICU admissions develop severe sepsis  
(27% of these die in ICU) – *Boots et al 2005 AIC 33:101-*



positive blood cultures ICU 2004  
*S aureus* > enterobacteriaceae > PA = enterococci

# GN bacteria are more lethal

- 100 patients: 140 consecutive episodes septicemia (135 bacterial and 5 fungal)
  - shock 19% (equal for ANC > 500)
- majority (>50%) isolates = GPC
- majority (83%) ICU transfers = GNR

# problems

- epidemiology not defined
  - traits (even at phenotypic level)
  - transmissibility and host-range
  - composite/ conditional nature

# proper epidemiology

- Infection control is about controlling spread of transmissible disease, including AR
- AR transmission occurs in the worst bugs (GNR) for the worst drugs (TIM/ GEN/ TOB/ 3GC)
- phenotypic screening is unreliable (routine methods miss things)

# predictable AR transmission

- *E coli/ K pn CTX/CAZ*>2 (2005-7; 60% comm.)
- *bla*<sub>CTX-M</sub> in 87% of ESBL
  - CTX-M-15 and -14 in var. strains and plasmids.
  - also new genes eg -62
  - no TEM ESBLs; only SHV-12 ESBL (+others)
- CIP-R >50%; trait not transferable (conditional)
- ESBL R to TIM, GEN, TOB
  - usu transferred (>60%)
  - predictable on context (~gene)

# limited NA targets

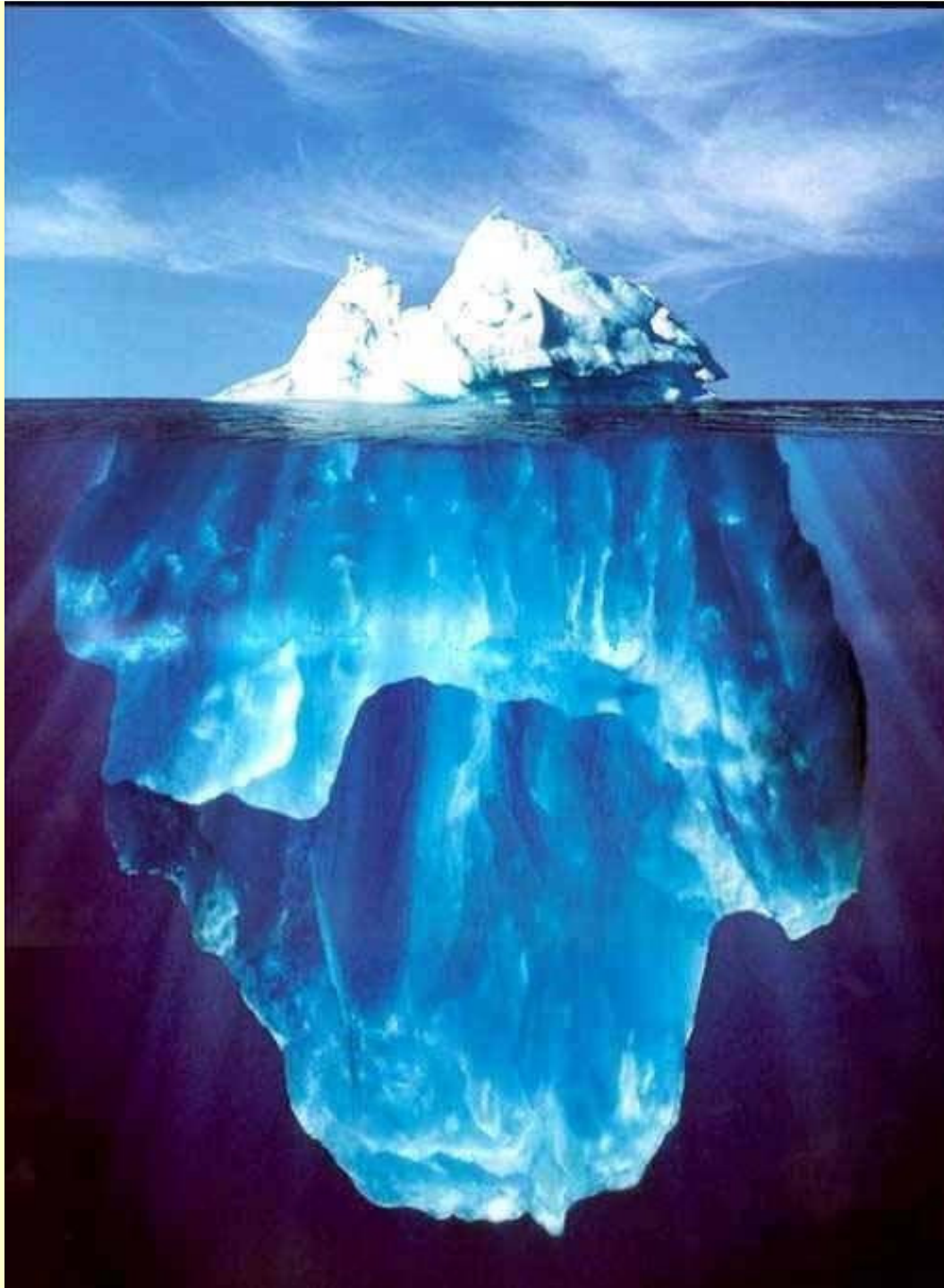
- *know your local epidemiology*
  - Gent-R in enterics: *aac3* and *aac6* types
  - pan-AG R: *rmtC* and *armA*
  - Tim-R in CTX/CAZ/TIM-R enterics :
    - ESBLs (CTX 85%, mostly -15 and -14)
    - MBLs (*imp4* 5%)
    - AmpCs (5%)
- *how local is local? (?regional/ temporal)*

the most common:  
*reasonable expectations*

- *S aureus*
  - the most important questions:
    - is it coagulase- positive?
    - is it resistant to methicillin?
  - when is it cost-effective?
    - rapid systems are better
    - are add-ons until proven ie cost extra

the most lethal:  
*reasonable expectations*

- Gram-negs
  - will it break through protocol combinations for septic shock?
    - aminoglycosides (gent/ tobra/ amikacin)
    - beta-lactams (APP- $\beta$ )
    - carbapenems
  - Infection Control implications?



recognising R potential  
= *infection control*  
= *direct intervention*

# 4 targets: MRSA/MRGN

*MRAcb*  
(*oxa23*)

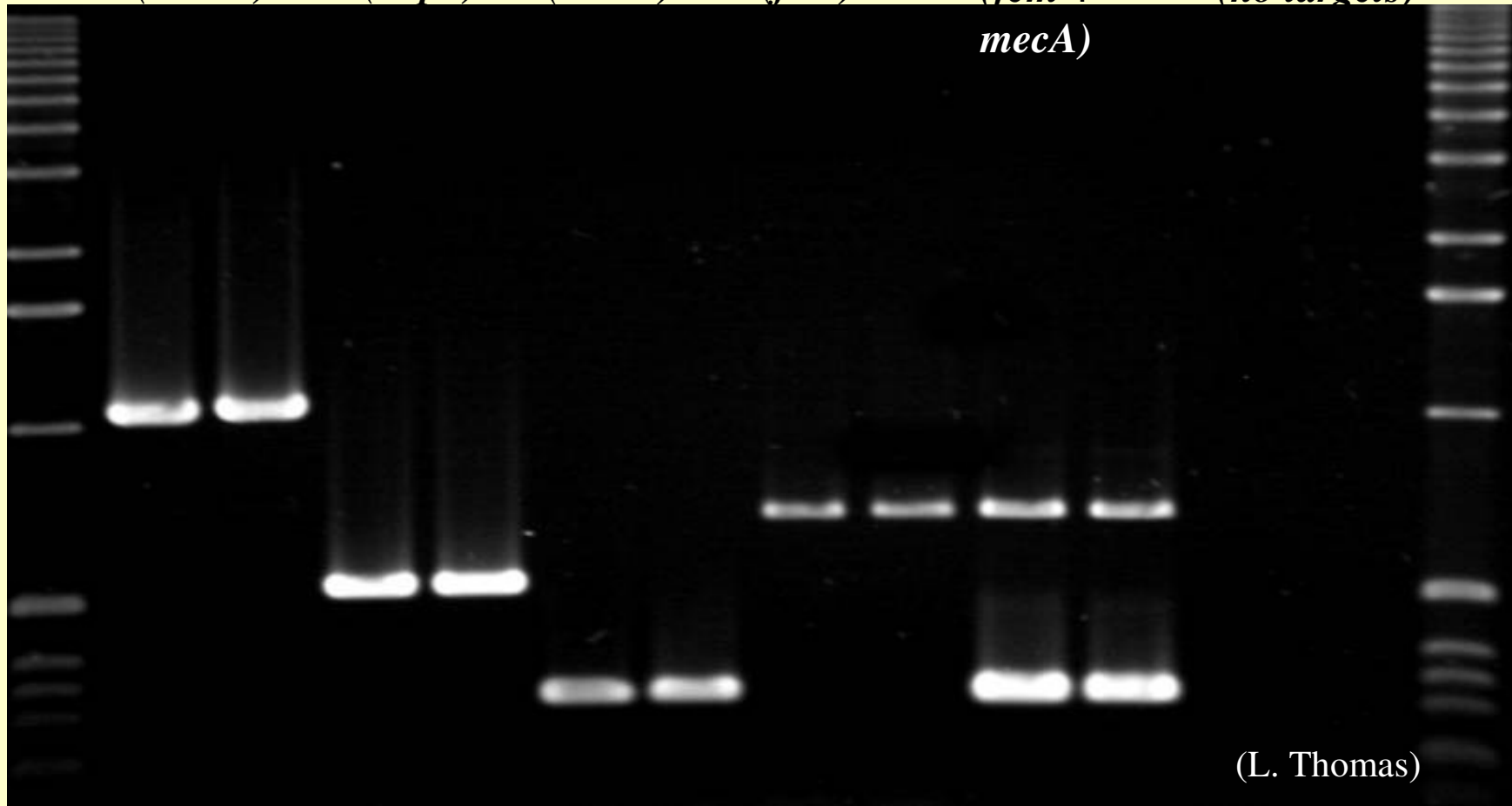
*MRE*  
(*imp4*)

*MRSE*  
(*mecA*)

*S aureus*  
(*fem*)

*MRSA*  
(*fem* +  
*mecA*)

*MSSE*  
(no targets)



# suitable devices

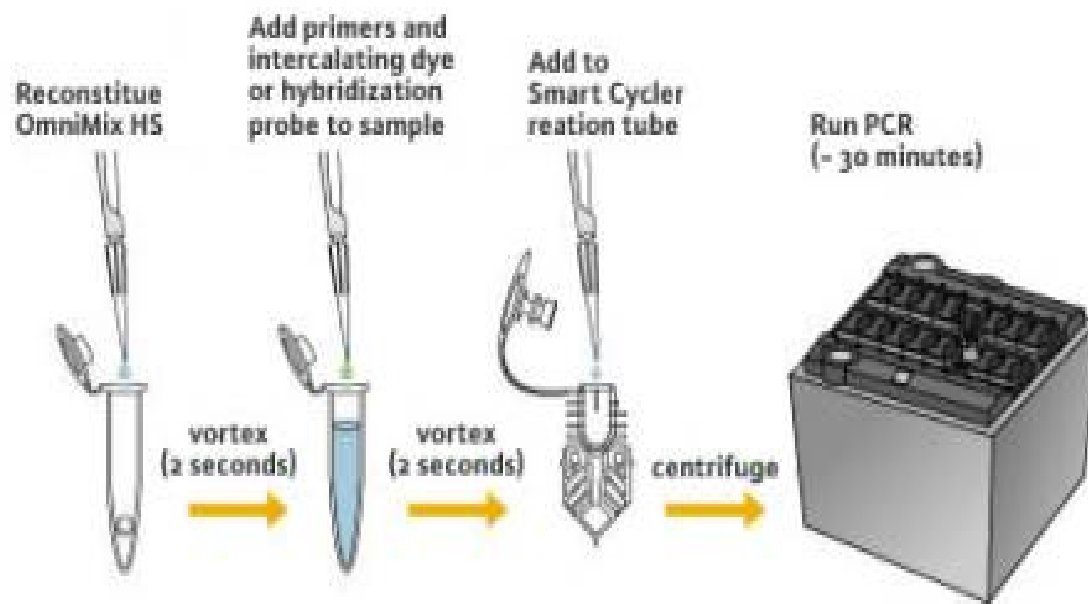
- fit lab workflow (as-needs basis)
- robust and reliable, ideally automated
- minimise specimen handling
- fast
- accurate
- multiplexed

# suitable devices

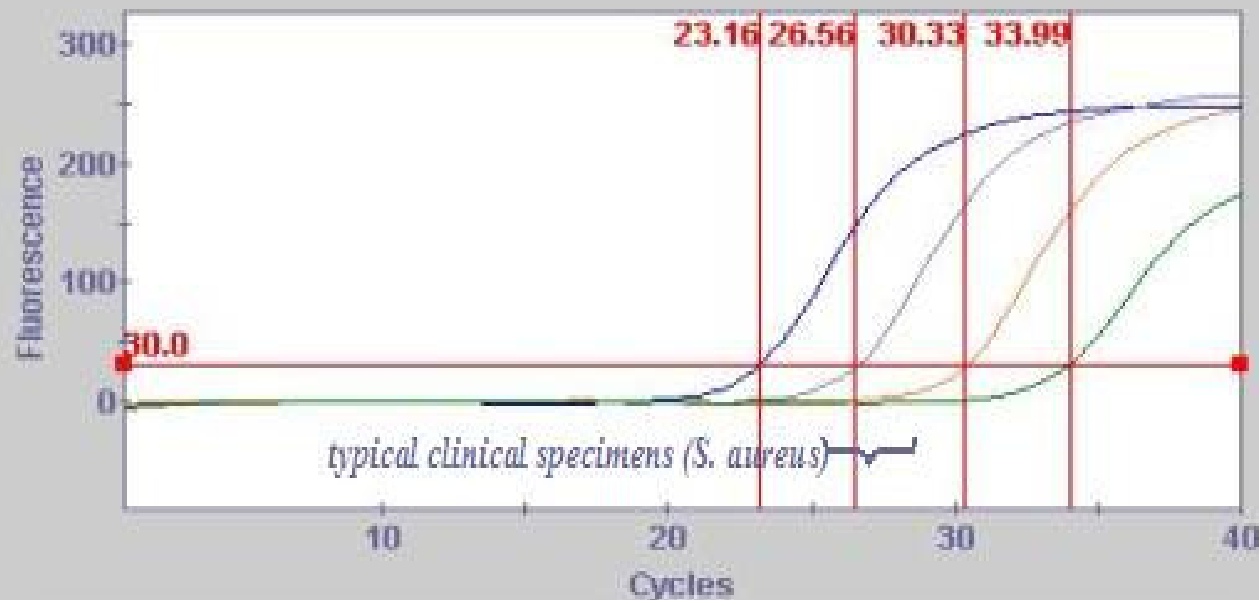
- random-access eg GBS, VRE, local MBL
- highly multiplexed diagnostics
  - predicting –R eg MEM, AMK
  - key idents eg MRSA, PA, VRE
- high-throughput screening
  - MRSA
  - MRSA, VRE, local GNR probs

# platforms

- NAA-based
  - random (single-) access devices
  - multiplexed systems
- “spot” tests (usu ab-based eg EIAs, aggn.)
- microarray platforms
- Luminex etc
- fluoroscopy
  - eg automated high-throughput TGLM – (FISH)



**Figure 1a.** An individual-well cycler uses a simple 3-step procedure for samples to be tested as needed (eg when a blood culture bottle signals positive), without special training or interruption to workflow. Specificity / sensitivity predetermined by PCR conditions (eg. *nuc* in Figure 1b)<sup>4</sup>.



Site ID	Protocol	Sample ID
A8	3.3.05	5
A10	3.3.05	0.5
A11	3.3.05	0.05
A12	3.3.05	0.005

**Figure 1b.** Automated detection of *S.aureus nuc* gene down to 0.005 ng/uL (Sample ID). Red verticals mark cycle numbers.

# SmartCycler (Cepheid)

- outperforms ref lab for sens and spec for (MR)SA
- simple and (relatively) cheap
- fast
  - <2h after blood culture signals
  - fast turnaround GBS in PROM saves \$\$\$\$\$

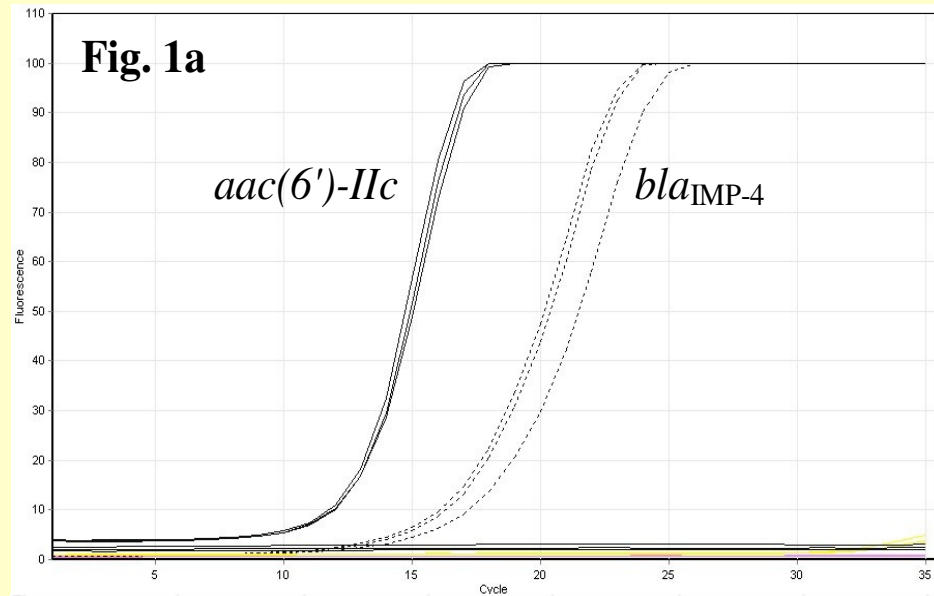
BUT

- limited multiplexing (<4) - (not its role)
- usu needs DNA extraction - (nb GBS system)

Table 4

Sensitivity and specificity of real-time PCR vs phenotype for identification of all clinical staphylococcal isolates, after resolution of discrepant results: the first (upper table) compares identification as methicillin susceptible, while the second (lower) compares identification as *S. aureus* or coagulase-negative

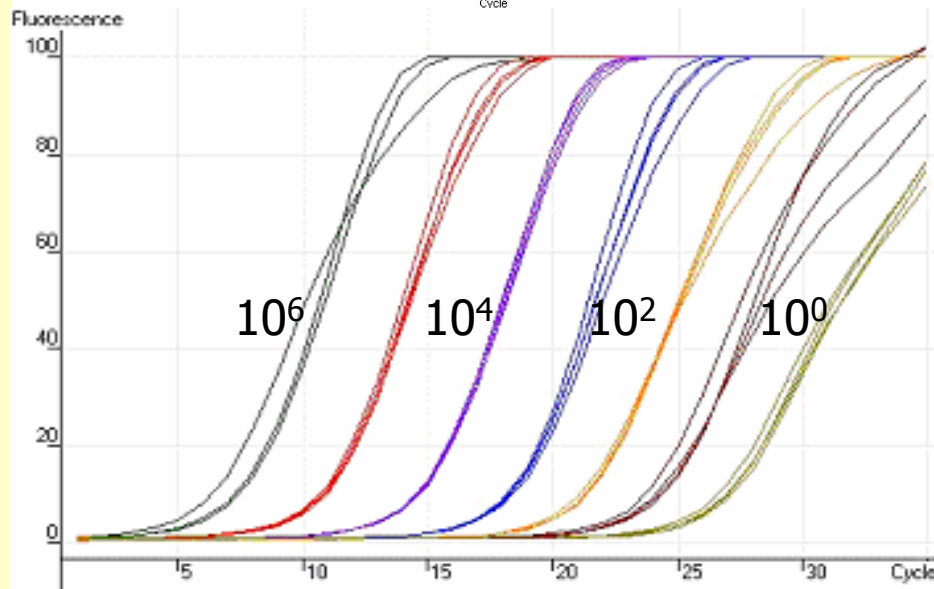
PCR result	Resistance phenotype (all staphylococci)	
	Methicillin resistant	Methicillin sensitive
<i>mecA</i> positive	64	0
<i>mecA</i> negative	2	54
Total	66	54
Sensitivity (95% CI)	64/66: 97% (89–100%)	
Specificity (95% CI)	54/54:100% (93–100%)	
PCR result	Laboratory identification (all staphylococci)	
	<i>S. aureus</i>	CoNS
<i>nuc</i> positive	43	0
<i>nuc</i> negative	1	76
Total	44	76
Sensitivity (95% CI)	43/44: 98% (88–100%)	
Specificity (95% CI)	76/76:100% (95–100%)	



**Fig. 1a: MT-PCR simultaneously measures multiple specific RNA or DNA targets.**

12 resistance genes were measured simultaneously (in triplicate): *aac(6')-IIc* and *bla<sub>IMP-4</sub>* are present in a single bacterial sample (unpub.).

*Fluorescence is shown on the vertical axis and cycle number on the horizontal.*



**Fig. 1b: MT-PCR is a sensitive and quantitative method**

Ct (cycle threshold) is a function of starting NA concentration: here, a 90 base DNA target ( $0.157 \mu\text{g}/\mu\text{L}$ ) is serially diluted to from  $10^6$  to  $10^0$  copies of template per reaction (2nd amplification round shown only). Water-only control gave no product (unpub.).

- ***Multiplexed tandem-PCR is a rapid method that simultaneously and accurately detects and quantifies multiple unique nucleic acid targets in individual isolates and in mixed populations.***

# MT-PCR (Corbett)

- multiple targets
- batched by disc/ assay
- (semi-)quantitative and HRM
- DNA and RNA
- relatively cheap

# staph screens

- MRSA
  - direct plating onto MAMSA: 47/58 at 48h
  - (MRSA1; MRSA2): 58/58 at 3h
  - after o/n incubn MAMSA broth: 58/58 at 56h
- MRSA neg
  - after o/n incubn MAMSA broth: 0/70 16h (36)
  - (MRSA1\*; MRSA2): 13/70; 3/81 at 3h
    - after o/n incubn MAMSA broth: (2/13\*) 2/70\* at 20h

# staph

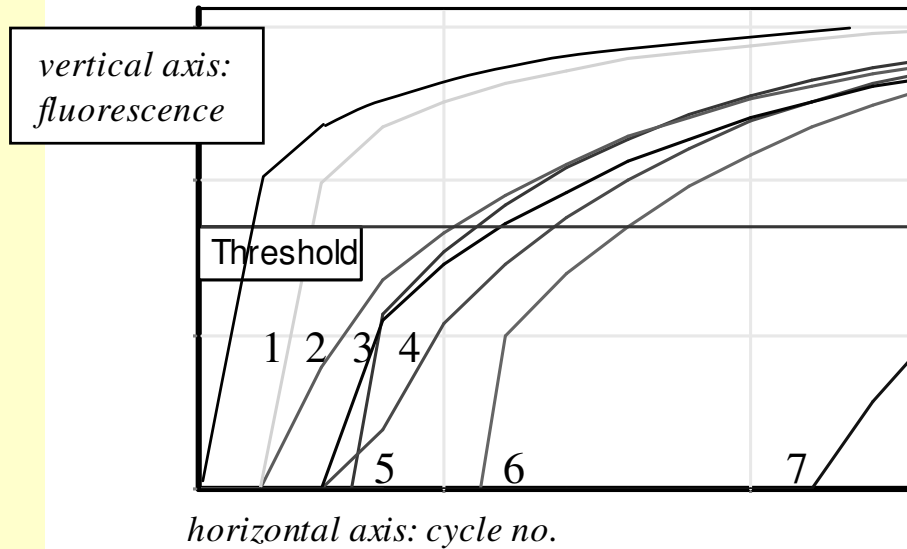
- direct plating:
  - insensitive (47/58)
  - specific (0/70)
  - slow (>36h)
- MAMSA broth o/n then plate:
  - sensitive (58/58)
  - specific (0/70)
  - very slow (56h)
- MRSA discs:
  - sensitive (58/58)
  - specific (2/70; 3/81)
  - quick
    - MRSA1 53/70 at 3h;  
68/70 at 20h
    - MRSA2 78/81 at 3h;  
(2 / 3 c/s negs IDI pos)
- ~revenue neutral on consumables

## resp (viral)

- 148 samples incl reference samples
  - fluA 20/20 (14 H-typed; 6 “fluA”)
  - H5 15/15 (extracts, SN, incl, v dilute specis)
  - RSV 11/11; RV 5/6; fluB 3/3; para3 2/2
- 85 negs at WM:
  - 6 fluAs; 11 RSVs; RV x 1; para3 x 3



specimen in a real-time assay [24]. In collaboration with the inventor (AI-Stanley), we have developed assays for respiratory infection<sup>a</sup>, for AR genes, and for particular bacteria (see Fig. 1b).



**Fig. 1b (MT-PCR):** 1.0 ng DNA from each of six templates was detected in MT-PCR, well before GAPdH internal control (#7), and without cross-reactivity (also in this disc were *gseA*, Strep-16S, *lytA*, *ply*, *vanC*). [JI and KS, unpubl.]

target	(test template)
1. <i>Pseudomonas spp</i> 16S	( <i>P. aeruginosa</i> )
2. Enterobacteriaceae 16S	( <i>E. coli</i> )
3. <i>Staphylococcus spp</i> 16S	( <i>S. aureus</i> )
4. <i>vanA</i>	(VR- <i>E. faecalis vanA</i> )
5. <i>nuc</i>	( <i>S. aureus</i> )
6. <i>vanB</i>	(VR- <i>E. faecium vanB</i> )
7. GAPdH	(spiked +ve control)

# three issues

- some results can't wait
- we perform worst where the need is greatest
- routine methods miss things

# three solutions

- random access rapid tests (in “real” time)
- highly multiplexed tests
- proper epidemiology

\$

# a simple strategy

- yearly surveillance of key R
  - comprehensive/ cheap *eg mPCR/RLB*
- check contextual relationships
  - fast/ automated *eg mt-PCR*
- decision points for critical diagnostics
- unit-specific screening for key R
  - *eg AMK/ MEM in GNs, MRSA/ VRE in GPs*

