New Microbiological techniques in bacteraemia
Improvements for the patient or improvements for the Microbiologist?

Jim Greig Consultant Microbiologist

Disclosures

Plagiarize,
Let no one else's work evade your eyes,
Remember why the good Lord made your eyes,
So don't shade your eyes,
But plagiarize, plagiarize, plagiarize...
Only be sure always to call it please, "research".

Nicolai Ivanovich Lobachevsky

solely for prosodic reasons

References

Buchanan et al Emerging technologies for the Clinical Microbiology Laboratory Clin Micro Rev 2014; 27: 783-822

Dixon P et al A systematic review of matrix assisted laser…. Eur J Clin Microbiol Infect Dis 2015; 34: 863-876

Cohen J et al Sepsis: a roadmap for future research Lancet Infect Dis 2015; 15: 581-614

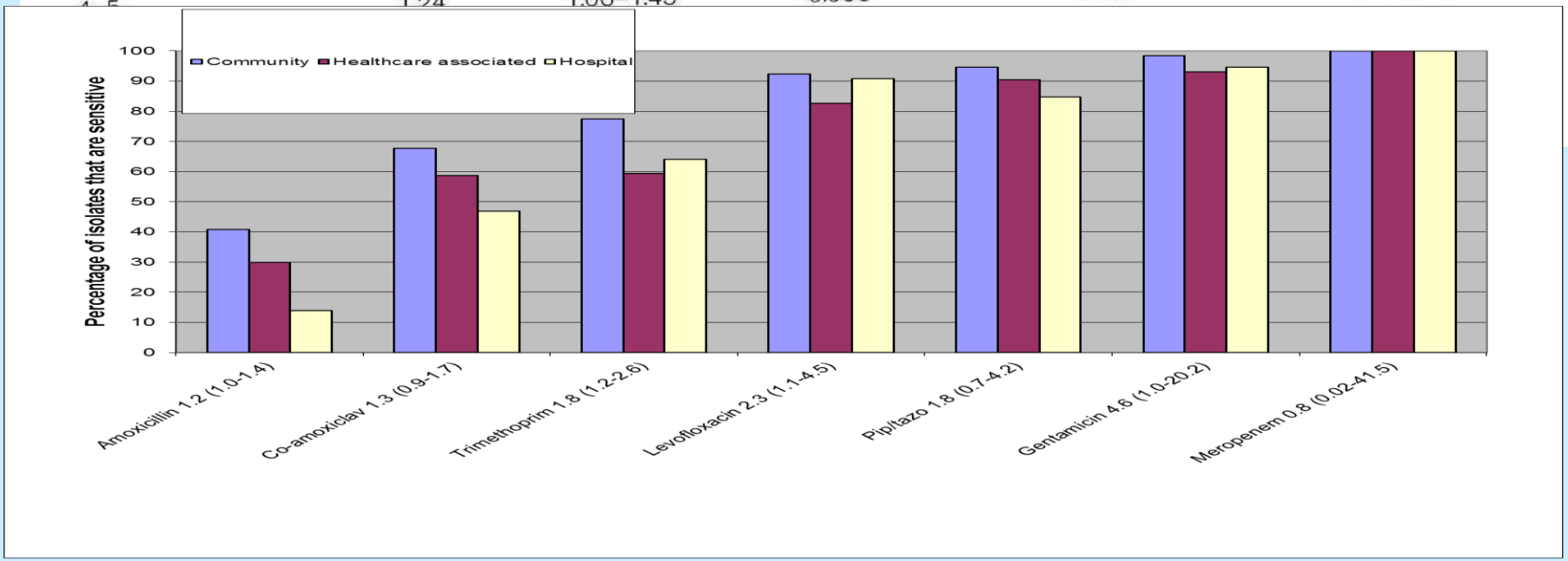
The nub of the issue

Most important first step is to use an appropriate initial regimen based on local epidemiology

Ferrer et al

TABLE 2. Adjusted Hospital Mortality Odds Ratio and Probability of Mortality for Time to Antibiotics Based on a Generalized Estimating Equation Population Averaged Logistic Regression Model

Time to Antibiotics (Hr)	OR[a]	95% CI	p	Probability of Mortality (%)[b]	95% CI
0–1[c]	1.00			24.6	23.2–26.0
1–2	1.07	0.97–1.18	0.165	25.9	24.5–27.2
2–3	1.14	1.02–1.26	0.021	27.0	25.3–28.7
3–4	1.19	1.04–1.35	0.009	27.9	25.6–30.1
4–5	1.24	1.06–1.45	0.006	28.8	25.9–31.7



Rapid and sensitive identification of antimicrobial susceptibility, both expressed and induced (rule out and de escelate)

Need the test to be sensitive (problematic)

Rapid and specific identification of what are contaminating gram positive bacteria (rule in and escelate)

Need the test to be specific (easy)

Always remember there may be the option to wait and see

German ICU (Lancet Infectious diseases October 2012)

Lower mortality and shorter length of stay

Correct OR for mortality with aggressive antimicrobial treatment was 2.5

Haemodynamically stable surgical patients with critical illness who are suspected of having an infection, waiting for objective evidence of infection before starting antimicrobials does not seem to worsen outcome

For critically unwell patients the ideal is broad spectrum antimicrobials covering all the likely virulent pathogens with prompt (and rigorous) de escelation

To allow this de escalation we need better and more rapid diagnostics

Naming the bug is only part (minor) of the issue, we need more rapid susceptibilities

- Is it MSSA, dispense with broad spectrum beta lactams
- Is it CNS, avoid escalation to a glycopeptide
- GNR, if a pseudomonas or Enterobacter, move from narrow spectrum beta lactams to broader spectrum

How many times will the name of the organism allow one to confidently de escalate at 24 hours in a critically unwell patient?

What can modern technology offer

Current timeframe

- 12-24 hours for Blood cultures to signal positive
- Further 24 hours for identification and sensitivities
- Maximum of 72 hours to get any unusual, special sensitivities
- Current/future, identification and sensitivities within hours

But in the real world……

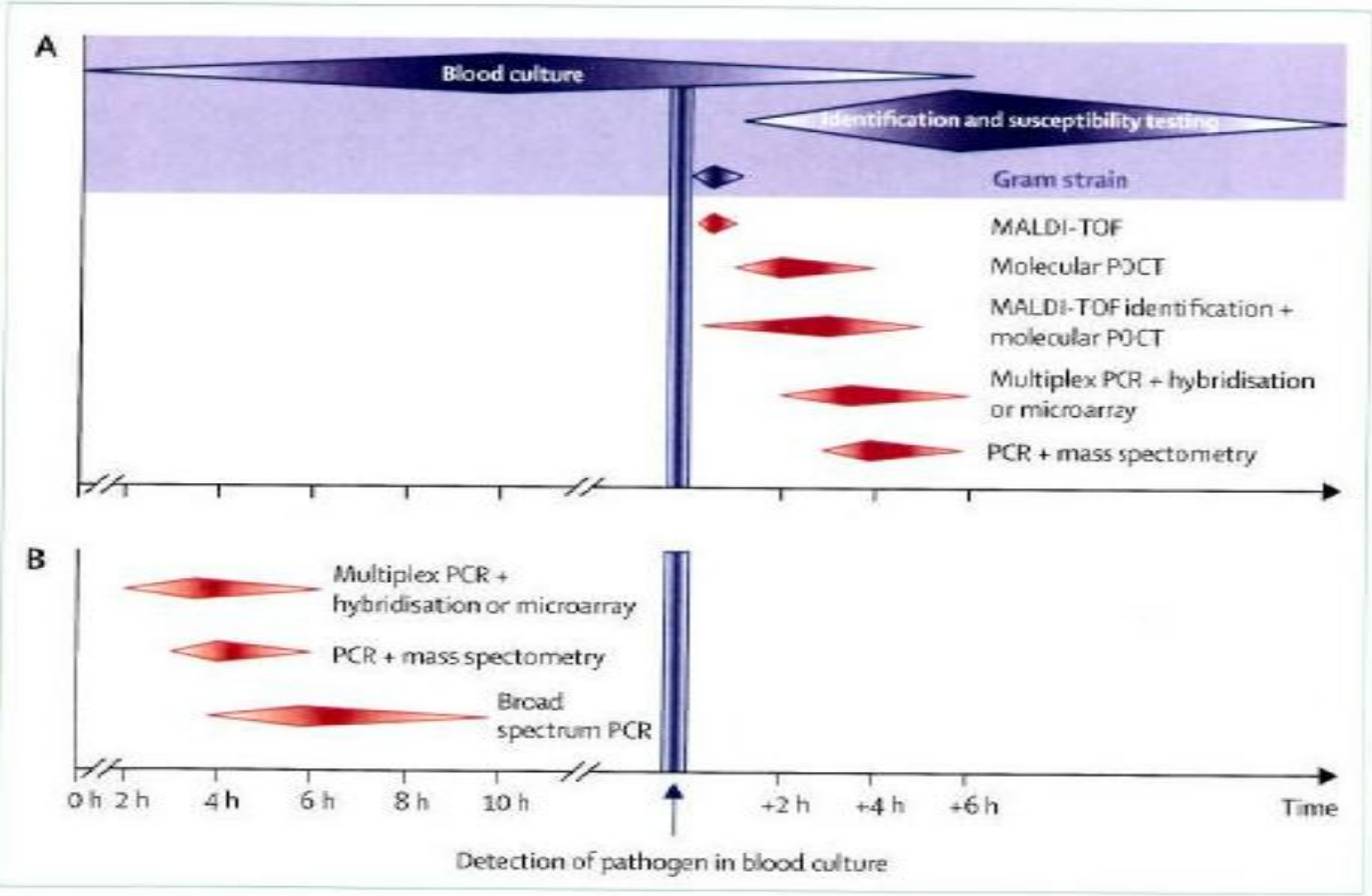
Time to:

- Recognise the urgency of the situation
- Take the blood
- Get the cultures to the laboratory
- Get the cultures incubating/on the on the machine
- Identify automated system as positive
- Inform the user of the culture

Improve time to laboratory before trying to improve time to bacteriological diagnosis

In hours	Ward et al 2015	Ronnberg C et al 2013	Lieven et al 2011	PHNT
Sample transport	3.7	4-10 hours (weekday and weekends)	12 (due to lack of 24/7 service)	12.3-7.7
Blood culture incubation	27.9 (incorporates inherent lab delays of batching)	10.3-14.5 (pre room temperature incubated, not pre incubated)	22	

Important to differentiate what is beneficial to the Microbiologist and what is beneficial to the patient



Emerging technologies

On positive blood cultures

Microarray

PCR MonoPCR with discriminating probes (Septifast)

Multiplex PCR (FilmArray)

PCR with electrospray detection

Mass spectrometry (MALDI)

In situ hybridisation (FIAH)

Next generation sequencing, whole genome analysis

NAATs

Benefits

- Rapid, as short as two hours
- Sensitive (sampling error)
- Specificity, (but need to know what you are looking for)

Disadvantages

- Costly, may be technically demanding
- Prone to contamination
- Interpretation in those simply colonised or successfully treated
- Minimal genetic material for typing
- Difficulties in measuring sensitivities and typing

Optimise current activity

Is the laboratory responsive to your needs
and are your needs likely to make a difference

Direct bile solubility
Short incubation DNAase
Direct phenotypic test
Early sensitivity reading (to identify resistance only)

Optimise your antibiotic stewardship, prompt IV/PO switch, use of review dates etc

Dryden et al 2012 One third on antibiotics could stop immediately and one in five on Ivs could switch immediately to orals

Laboratory attributes of modern technology

Does it fit in with the processes and practices in the laboratory (skill mix)

Open or a closed platform

On demand or batched

Is it a sample to result format or a hands

on one

Are there streamlined processes to use the data

Ideal is on demand sample to result integrated with the Trust computer system

Squirt and forget

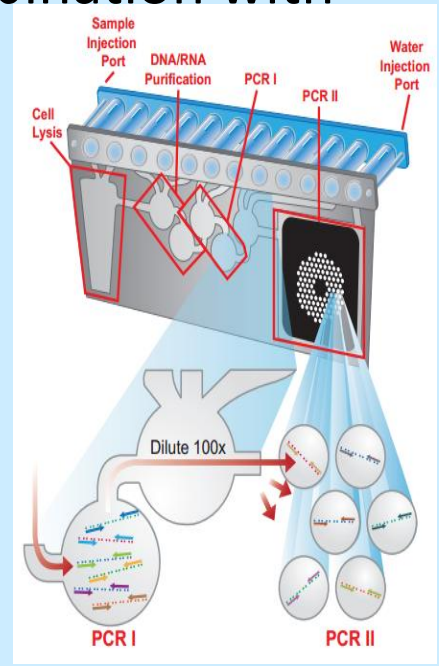
Examples are used to highlight the technology only

There are many tests on the market, this is in no way an endorsement of any!

Post culture PCR techniques

Monoplex targeted Identification staphylococcal species and presence of methicillin resistance

Multiplex with liquid probe identification or combination with microarray



Filmarray BCID

8 positive, 11 gram negative, 5 yeasts pathogens and resistance markers (mecA, van A/B, KPC)

Multiplex pouch kit

Expensive and single test per machine capability

Only identifies what it looks for (90% common pathogens)

Sensitivity and specificity >95%

Difficulties in handling polymicrobial cultures (5-10% of all cultures)

Microarray

Once a blood culture signals positive considerable microbial nucleic acid

Use nucleic acid hybridisation technology

Low densitiy chips (100 probes) high density (millions of probes)

Commercial system is **Verigene**

Performance in the region of >95% for in product ID and 90% total

Extensive resistance profiles

Nine gram negative, 13 gram positive and Mec A, Van, six main groups of extended spectrum beta lactamases

Test sensitivity and specificity >95%

Real time impact of multiplex PCR and microarrays

The name is interesting any be informative

Want to know what the bug is sensitive to (resistance)

Bork 2015 Verigene and AST, theoretical advantage in time to optimal therapy only

No carbapenem resistant pyos correctly identified

Ward 2015 Compared Verigene, FA and MALDI side by side with modest (5% of patients) benefits of AST

Ljungstrom 2015 Time to ID of Prove It (PCR/microarray combo) was only 1-2 hours more rapid than (delayed!) MALDI

Bork et al

	Effective antibiotic treatment		Optimal antibiotic treatment	
Time of BC-GN report	**Hazard ratio 95% CI**	**Mean difference**	**Hazard ratio 95% CI**	**Mean difference**
3 h	1.11–1.60	4.8 h	2.00–3.12	22.5 h
6 h	1.09–1.54	4.4 h	1.95–3.04	21.1 h
12 h	0.98–1.33	3.7 h	1.86–2.89	18.3 h

Pick those low hanging fruits

All times in hours	A trial 2015
Sample transportation	3.7
blood culture incubation	27.9 (incorporates inherent lab delays of batching)
Gram stain result	7.5
Species identification (MALDI)	18.2
Sensitivities	20.3
Total to ID	57.3
Total to sensitivity result	69.4

Mass spectrometry

Vaporise or ionise pathogens into pieces of differing size and charge (mass to charge ration)

Accelerate across a vacuum and measure time of flight, related to m/z ratio

Produce a MS profile and compare with a bank of pre tested isolates and identify the best match

Relies on very controlled laser

Ionisation and a large and

diverse spectral reference

library

Uses

Rapid identification of pathogens directly from solid culture

Rapid identification of pathogens from liquid (including Blood) cultures

- Inexpensive consumable, large capital cost outlay
- Result within minutes of loading
- Diagnostic accuracy is 90-95%,
- Identification of difficult pathogens

  eg fungi and mycobacteria, hindered

  by library diversity

Clinical (not laboratory benefits)

Limit of detection estimated 150,000 organisms, unable to separate mixed cultures (but will then give unacceptable profile, it fails safe

Used to identify organisms from positive (incubated) blood

Direct urine analysis using centrifugation concentration, 92-95% gram negative sensitivity in pure heavy culture

Susceptibility testing

MALDI relies on the bacterial phenotype not targeted genetic testing

MS profile of resistance mechanism is below level of detection

Methods

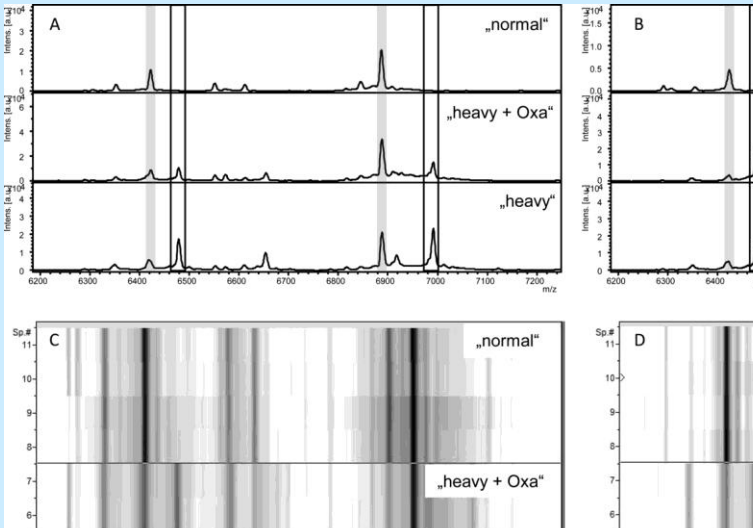
If resistance due to antibiotic destruction, brief incubation of microbe with target antibiotic will lead to no antibiotic MS profile

Incorporate stable heavy amino acid

isotope into growing bacteria, easily

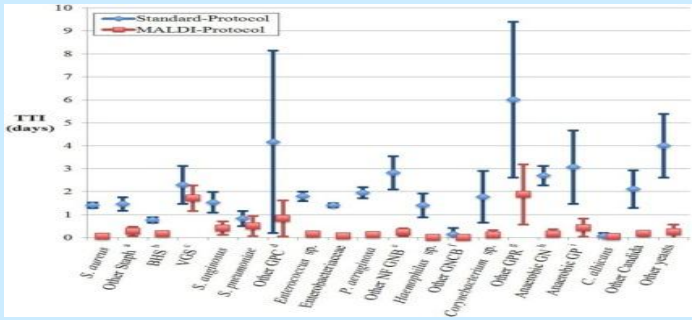
identifiable from Wild type by

MMS profile



Real time benefits of MALDI

Clearly speeds up time to identification especially if performed direct from positive culture



How can this benefit in the absence of susceptibilities

Dixon 2015 systematic review of time to <u>identification</u>

10 studies identified, none were RCTs (in progress), all before and after studies

Average improvement time to ID (over comparator) was 33 hours*

Four have looked specifically at AST

Perez and Huang showed a benefit but controls inadequate

Vlek et al demonstrated that 50% of patients were not on active antibiotics, at 24 hours use of MALDI improved this to 75% (64% without MALDI)

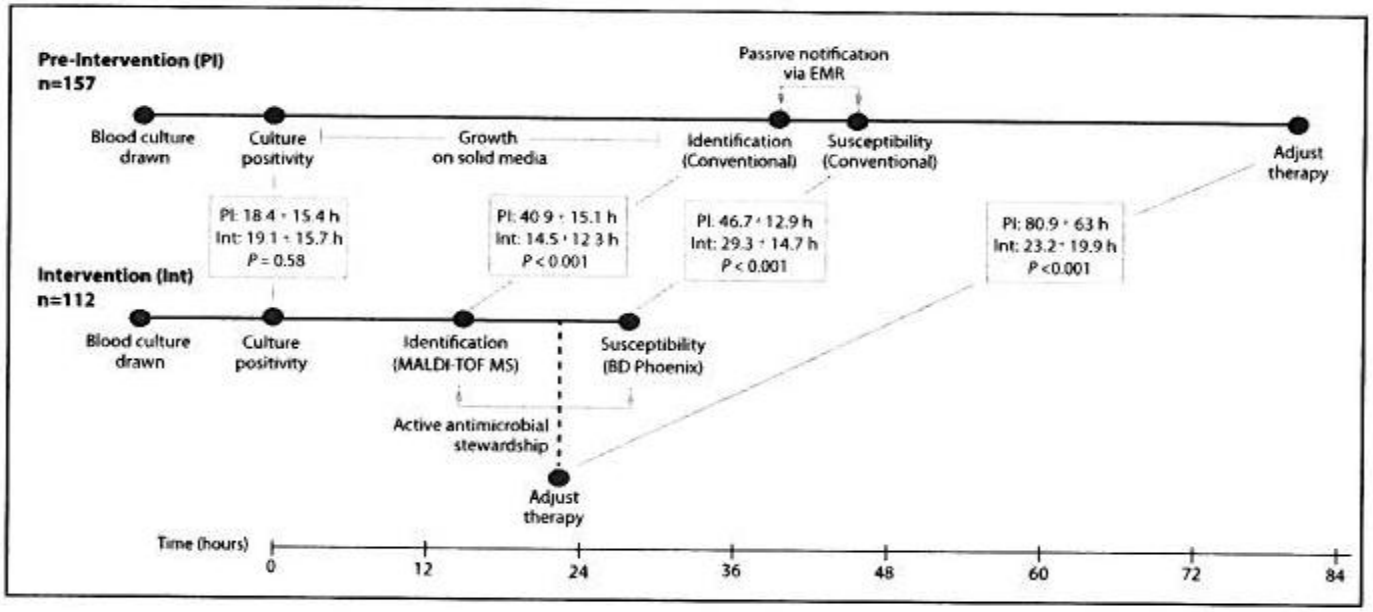
Clerc showed in gram negative bacteraemia gram stain led to intervention on 21% occasions, MALDI led to intervention in a further 39% of the rest

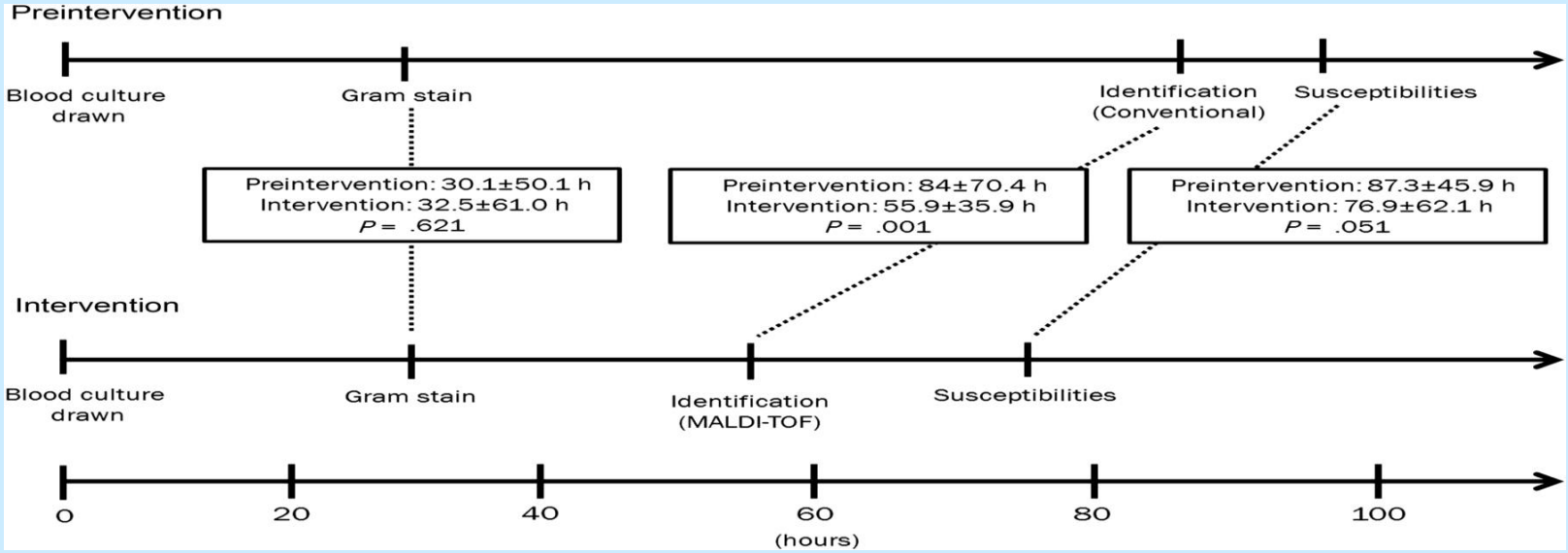
Further 15% of possible interventions

Risk factor for not taking up recommendation was ICU acquisition

Table 3. Impact of Sequential Gram Stain and MALDI-TOF Reporting

Impact of the Sequential Reporting	N = 202
Gram stain	42 (20.8)
Streamlining	16 (7.9)
Spectrum broadening	16 (7.9)
Introduction of empirical antibiotic therapy	10 (5.0)
MALDI-TOF MS	71 (35.1)
Streamlining	22 (10.9)
Spectrum broadening	31 (15.3)
Introduction of focused empirical antibiotic therapy	18 (8.9)


Pre-Intervention (PI)
n=157
Passive notification via EMR
Blood culture drawn
Culture positivity
Growth on solid media
Identification (Conventional)
Susceptibility (Conventional)
Adjust therapy
PI: 18.4 ± 15.4 h
Int: 19.1 ± 15.7 h
P = 0.58
PI: 40.9 ± 15.1 h
Int: 14.5 ± 12.3 h
P < 0.001
PI: 46.7 ± 12.9 h
Int: 29.3 ± 14.7 h
P < 0.001
PI: 80.9 ± 63 h
Int: 23.2 ± 19.9 h
P <0.001
Intervention (Int)
n=112
Blood culture drawn
Culture positivity
Identification (MALDI-TOF MS)
Susceptibility (BD Phoenix)
Active antimicrobial stewardship
Adjust therapy
Time (hours)
0
12
24
36
48
60
72
84


Preintervention
Blood culture drawn
Gram stain
Identification (Conventional)
Susceptibilities
Preintervention: 30.1±50.1 h
Intervention: 32.5±61.0 h
P = .621
Preintervention: 84±70.4 h
Intervention: 55.9±35.9 h
P = .001
Preintervention: 87.3±45.9 h
Intervention: 76.9±62.1 h
P = .051
Intervention
Blood culture drawn
Gram stain
Identification (MALDI-TOF)
Susceptibilities
0
20
40
60
80
100
(hours)

Pre culture diagnosis

Typical bacteraemia has 1-10 cfu per ml of blood but levels of genomic DNA 2-3 log order greater

Pre incubate then PCR multiplex eg FilmArray

Half of ultimately positive blood cultures FA reactive on receipt in lab

Spiked bottles all FA positive at 5 hours pre

Longer transport time greater likelihood to be positive

Pre culture diagnosis

Specifically designed direct PCR assays

Suboptimal sensitivity (50%) and pick up more pathogens than conventional culture (what is the Gold Standard)

Septifast (just an example) is an open platform, manual system considered of high laboratory complexity, requires no pre incubation

No resistance information available

Sensitivity, specificity	62-74% and 84-89%
NPV and PPV	93-96%, 19-32%

Electrospray MS

Simple means of identifying PCR amplicons

Simultaneous multiplex PCR and targeted detection

More discriminatory than MALDI or probe amplicon detection, able to handle polymicrobial samples

Typical direct blood sensitivities in the region of 50%, can achieve higher with optimised sample preparation

Bacconi JCM 2014

Spiked specimens showed limits of detection of 16cfu/ml

Clinical study showed good agreement (sensitivity 17/20 and 100% specificity) than culture and twice as many positives

Could not replicate with direct Sanger sequencing

Increased sensitivity to 83%

Are we there yet

We are in the laboratory when it comes to rapid identification from positive cultures

Much of the genetic assays are probably already surpassed by MS techniques

Pre culture genetic testing remains disappointing and difficult to interpret

On the horizon is electrospray PCR which may be overtaken by whole genome sequencing