The Role of Rapid Diagnostics in Preventing Healthcare Infection

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Declaration

The views expressed are of a professional but personal nature and not necessarily those of the RCSI & Beaumont Hospital, Dublin.

I have recently received research funding from Pfizer & Astellas. I have also provided professional advice or education to Cepheid & Pfizer.

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Objectives

- Provide a brief overview of healthcare-acquired infections (HCAI) & the specific current challenges
- Discuss current limitations in the techniques used to detect HCAI
- Explore the role of whole genome sequencing & other emerging technologies in detecting & defining spread
- Outline when & where such technologies will be used

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Importance & Trends in HCAI

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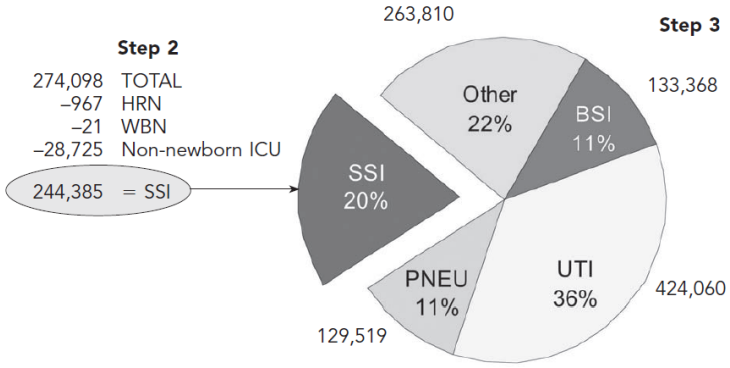
HCAI in USA, 2002

- **Multiple datasets: 1.7m with HAI, 155,668 deaths & 98,987 due to HCAI; Mortality highest for pneumonia & BSI**

Figure. Calculation of estimates of health care-associated infections in U.S. hospitals among adults and children outside of intensive care units, 2002



Pub Health Rep 2007; 122: 160-166

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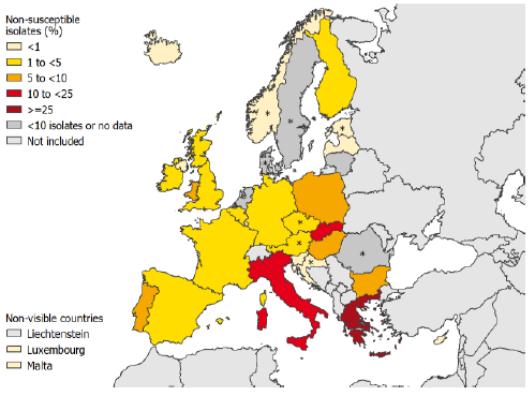
HCAI in Europe, 2011-2012

- **1,000 hospitals in 30 countries**
- **5.7% overall; 19.5% in ICU**
- **RTI 23.5% > SSI 19.6% > UTI 19% > BSI 10.7%**
- **32.7% on antibiotics**

d. Carbapenem-non-susceptible *Enterobacteriaceae*



ECDC, July 2013

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Current Limitations & Needs into the Future

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Unlike with other laboratory diagnostic approaches, e.g. haematology, a result is not available in many cases for 1-2 days.

Therefore appropriate therapy may be delayed. Over-treatment may be given & transmissible multi-drug resistant infections may have resulted.

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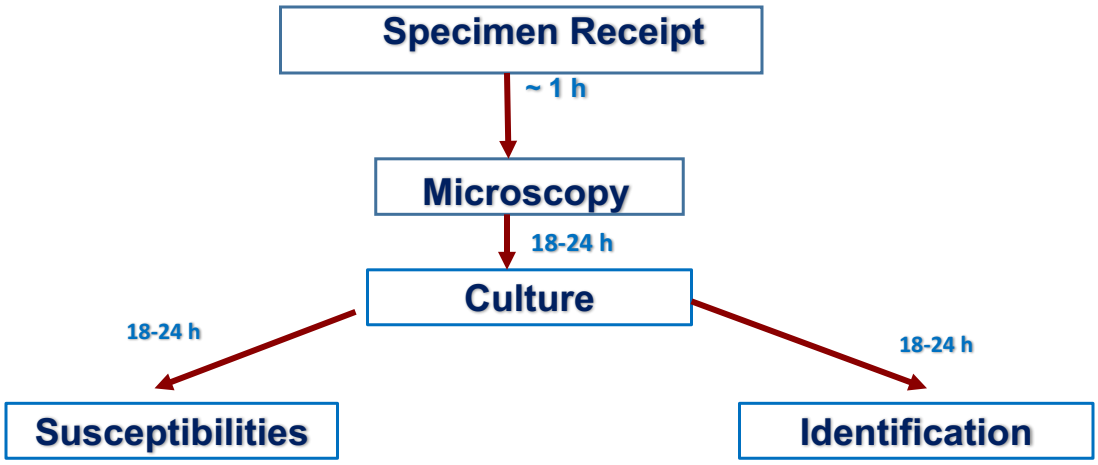
Bacterial Diagnosis - Traditional



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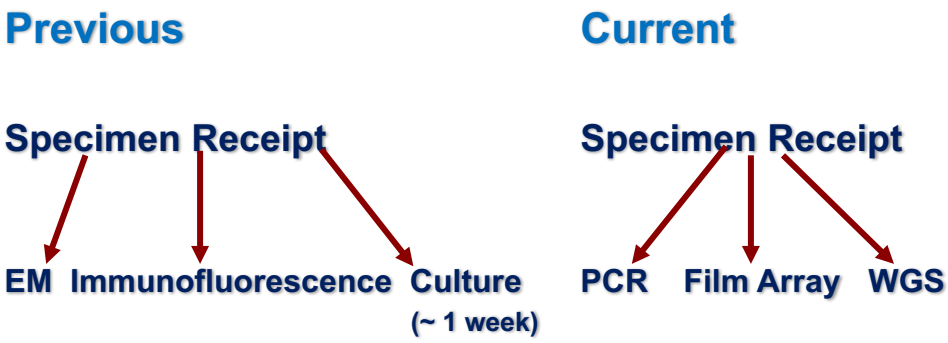
Viral Diagnostics



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Priorities

I. Acute, life-threatening infections such as bloodstream infections (BSI), meningitis

II. Multi-antibiotic resistant bacteria

III. Emerging, opportunistic & potential causes of HCAI, e.g. Zika virus, Ebola virus, astrovirus

IV. Fast tracking of HCAI spread within & between hospitals

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What the future may bring

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Current & Emerging Technologies

Current

PCR

Mass Spectrometry

Whole genome sequencing (WGS)

Emerging/Evolving

Electronic nose devices
(volatile organic compounds)

Infra-red spectroscopy

Microfluids

Ann Lab Med 2013; 33: 14 -27

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Diagnostic Methods & Time Required

Diagnostic Method	Time for Pathogen Identification
Microscopy	Morphology in minutes
Gram stain	General category in minutes
Culture and phenotypic biochemistry on/in artificial media (bacterial, mycobacterial, fungal)	Days to weeks
In vitro antimicrobial susceptibility	Days to weeks
Acute and convalescent antibody	Weeks
Monoclonal antibodies	Hours
Antigen detection	Minutes to hours
Real-time polymerase chain reaction for microorganisms and drug resistance genes	One to several hours
Mass spectrometry	Seconds to minutes, after growth on/in media

Clin Infect Dis 2013; 57: S139-70

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Overview of Clinical Bacteriology

1. Molecular panels for BSI – emerging
   - May not detect all microbes if mixed infections
   - *mec*A may be from *S. aureus* or coagulase negative staphylococci
   - 24h staffing
2. Rapid identification & susceptibility testing
   - down from a day to hours or even minutes
3. Metagenomics (WGS)
   - human DNA "subtracted" & leftover DNA analysed

Mayo Clin Proc 2016; 91: 1448-1459

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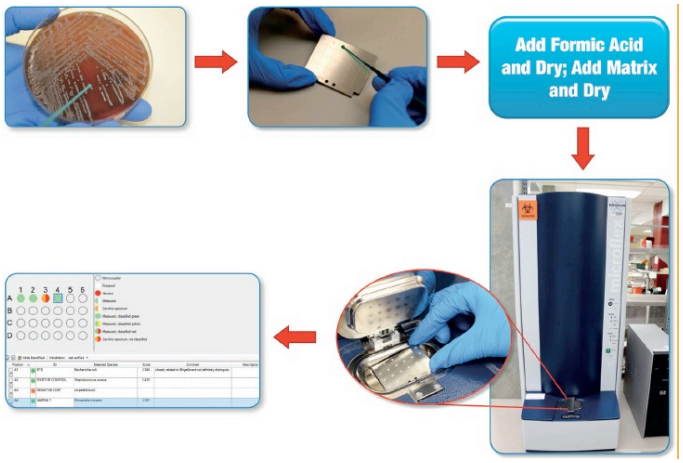
Overview of Clinical Bacteriology



What is the impact of this & other new emerging technologies on mortality, length of hospital stay & BSI duration?

Mayo Clin Proc 2016; 91: 1448-1459

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Infectious Diseases Society of America (IDSA) Policy Paper

Federal priorities & incentives for tests

a) Directly on accessible specimens such as blood
b) Exclude infection, e.g. ≥ 98% negative predictive value
c) Incorporate biomarkers that indicate host response
d) Panels for clinical syndromes, e.g. CNS infections
e) Drug resistance
f) Point-of-care
g) Improved outbreak detection

Clin Infect Dis 2013; 57: S139-70

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IDSA Policy Paper

Where time makes a difference

- HIV resistance & anti-viral choices
- Unrecognised/unculturable organisms, e.g. HCV, *Tropheryma whipplei*
- Methicillin-susceptible or resistant *S. aureus*
- Middle East Respiratory Syndrome coronavirus (MERS-CoV)
- Genotyping, e.g. HPV 16 & 18 associated with neoplasia & in-outbreak investigation

Clin Infect Dis 2013; 57: S139-70

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Point-of-Care Testing (POCT)

"Testing of specimens, whether in a laboratory or not, close to the patient, e.g. doctor's office, 24h a day with a result within 2-4h"

Simple, safe & quick tests
Minimal equipment required
Cheap or cost effective
Less specialised equipment
Bedside or satellite laboratory

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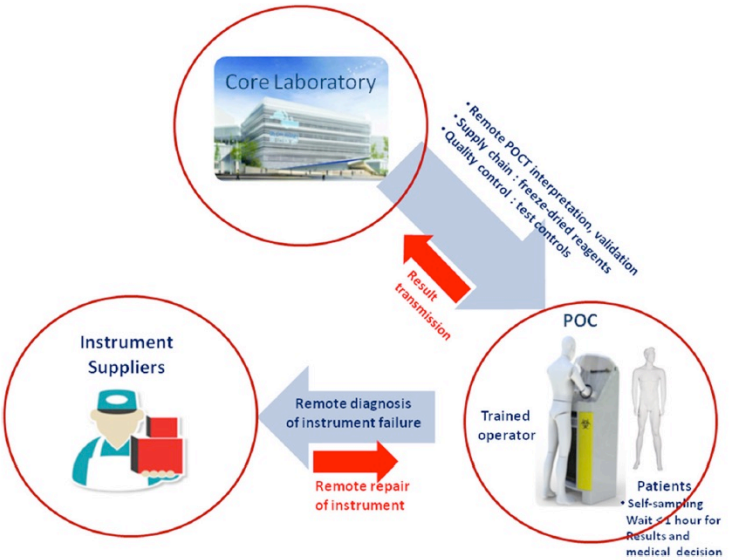
POCT Set Up



Clin Microb Rev 2016; 29: 429-447

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POCT Options

Antigen assays for syndromes

e.g. diarrhoea-rotavirus, adenovirus, *Clostridium difficile, Campylobacter* spp.

Cheap, easy to use but low sensitivity such as 60%

Real-time PCR (RT-PCR)

e.g. meningitis – *Neisseria mengitidis*, *Streptococcus pneumoniae,* enterovirus, herpes simplex virus, varicella-zoster virus

Variations accordingly to geography, patient categories (e.g. Cryptococcus) & possibly cheaper in resource-poor countries, e.g. TB

Clin Microb Rev 2016; 29: 429-447

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Rapid BSI Detection

Challenges from +ve blood culture

- PCR inhibitors
- High quantity of non-microbial nucleic acids
- Contaminated DNA
- DNA from dead microbes
- Not a "catch all" approach

Other Issues

- Choice of probe dependent on Gram stain
- Bacterial load is usually 10^6 - 10^8
- Turnaround time (TAT) of 1.5 – 4 h

Clin Microb Infect 2015; 21: 313-322

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Available Rapid BSI Systems (adapted)

System	TAT	Organism detected	Sensitivity & Specificity
FISH (UC)	1-3h	Up to 4 Gr - & 5 fungi	81-100% 90-100%
Microarray	2.5 -3.5h	Up to 60 bacteria & 13 fungi	81-100% 95%
Multiplex – PCR	1-2 h	8 Gr +ve, 11 GR-ve, 5 fungi	91-100% 95-98%
Mass-spectrometry	< 1h	<1,000	76-99%

Clin Microb Infect 2015; 21: 313-322

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SepTec™ Technology for BSI in ICU

- Microfluidic device that uses 2 ml
- Detects <10 colony forming units in < 25 minutes
- Categories as → Gram positive
  Gram negative
  Fungal

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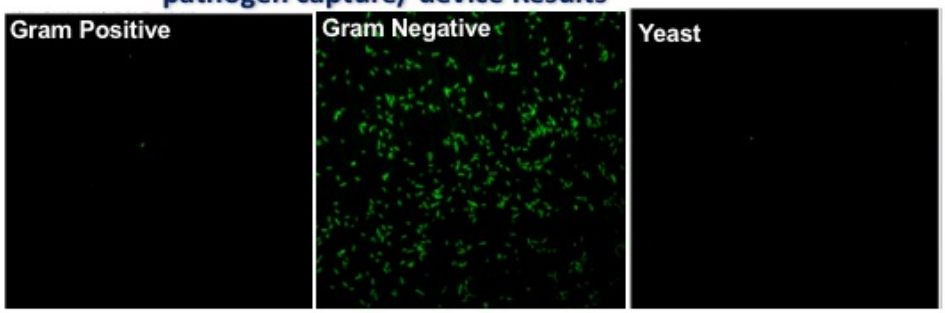
Detection Example – Post Blood Culture

Blood (Post-Culture) Patient Sample D008

Confocal Fluorescence Imaging currently used as additional tool to confirm pathogen capture/ device Results



High Capture Efficiency from Whole Blood for 10 minute incubation time

Rapid RTI Diagnosis

Pneumonia (available) but MERS-CoV & TB (POCT) needed

	Time to result	Type of technology	Targets	Sensitivity	Specificity
Cepheid Xpert MRSA/ SA SSTI[62]	1 h	Automated sample preparation of respiratory specimen, real-time PCR and detection using molecular beacon technology	MSSA and MRSA	99·0% compared with quantitative culture of endotracheal aspirates	72·2% compared with quantitative culture of endotracheal aspirates
Curetis Unyvero Pneumonia P50 Test[63]	4 h	Multiplex endpoint PCR and amplicon detection by hybridisation to oligo probes spotted on membrane arrays direct from respiratory samples	Detection of 17 bacterial and fungal pathogens in addition to 22 antibiotic resistance genes	80·9% overall; target specific values 50–100%	99·0% overall, target specific values 72·3–100%
Biofire Filmarray Respiratory Panel[64,65]	1 h	Pouch format comprising nucleic acid extraction, and nested PCR from nasopharyngeal swabs	20 targets including respiratory viruses, *Bordetella pertussis*, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*	84–100%	98–100%

MSSA=methicillin-sensitive *Staphylococcus aureus*. MRSA=methicillin-resistant *S aureus*. SSTI=skin and soft tissue infection.

Lancet Infect Dis; 2014: 14: 1123-35

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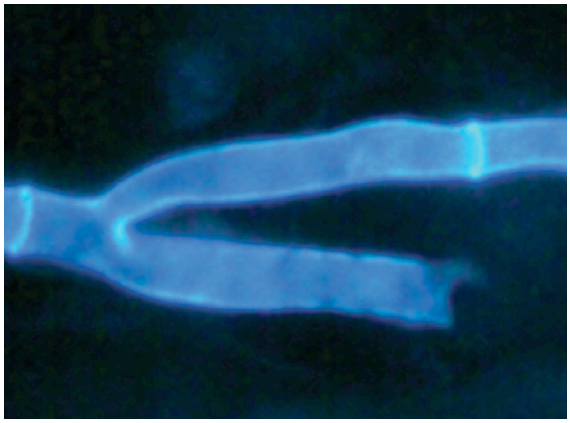
Rapid Testing & Fungal Infections

- Mainly still microscopy, culture & histology
- Galactomannan & β-D-glucan screening of serum
- PCR in combination for aspergillosis but often systems are not validated
- MALDI-TOF assists in identification



Lancet Infect Dis 2015; 15:461-74

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Carbapenamase-Producing Enterobacteriaceae (CPE)

- A global problem, a national problem in many countries & a local one
- Hospital & community
- ≥ 1 mechanism, multiple genus & species
- Traditional methods cumbersome & slow

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ESBL/CPE –Rapid Biochemical Tests

Test	Mechanism	Turnaround time	Comments
NDP	Cefotaxime hydrolysis	<1 hour	>98% S&S
Carba NP	Imipenem hydrolysis & change in pH	2 h	Low sensitivity to OXA-48
Blue-carba	Bromothymol blue indicator	Faster as no extract process	Better for OXA-48

Infect Dis Clin N Am 2016, 323-45

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CPE – Molecular & Other Tests

- Ideally want to target
  CTX-M, TEM, SHV, KPC, IMP, VIM, NDM, OXA
  e.g. hyplex SuperBug, Curetis AG, GeneExpert
- In the future, the options will be

WGS

Micro-arrays – detect multiple genes & can be updated, e.g. Alere

Modifications to MALTI-TOF MS

Microfluidics & nanotechnology, lab-on chip

Infect Dis Clin N Am, 2016; 323-345

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Rapid Screening for MRSA

- Beaumont Hospital study of 462 (ward, emergency department & ICU) patients during 3 periods
- 22-33% MRSA +ve
- 27% not screened if culture used & 11% if PCR used ($p<0.01$)
- 24% of patients pre-emptively isolated without PCR compared to 7% with PCR ($p>0.001$)

Infect Control Hospital Epidem 2010; 31: 374-381

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Does rapid detection of BSI & or identification of cause +/- resistance markers make a difference?

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SeptiFast Blood Culture Detection

TABLE 1 Pathogens detectable using LightCycler SeptiFast test

Gram-negative bacteria	Gram-positive bacteria	Fungi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella (pneumoniae/oxytoca)	CoNS[a]	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus* species[b]	*Candida glabrata*
Proteus mirabilis	*Enterococcus faecium*	*Candida krusei*
Acinetobacter baumannii	*Enterococcus faecalis*	*Aspergillus fumigatus*
Pseudomonas aeruginosa		
Stenotrophomonas maltophilia		

a Single probe detects a group of staphylococcal pathogens including *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*.
b Single probe detects a group of streptococcal pathogens including *Streptococcus pyogenes, Streptococcus agalactiae* and *Streptococcusmitis*.

Health Tech Ass 2015; 19 (35)

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Rapid Detection of HCAI BSI in Critical Care with Multi-Pathogen PCR

- SeptiFast real time PCR compared to blood cultures
- 25 pathogens detected in single blood samples
- 2129 citations →37 studies
- Study quality variable with bias a possibility
- 59% sensitivity & 89% specificity: better rule-in than rule-out potential
- SeptiFast unlikely to result in sufficient diagnostic utility of suspected sepsis-related HCAI

Health Tech Ass 2015; 19 (33)

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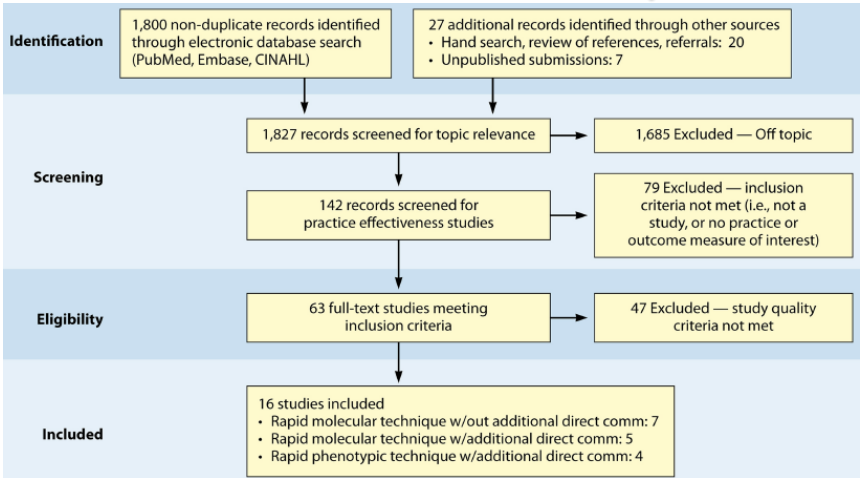
Effectiveness of Improved Timelines of BSI Results

Compared rapid phenotypic tests & additional communication, rapid molecular tests & additional communications & rapid molecular tests only



Clin Microb Rev 2016; 29: 59-103

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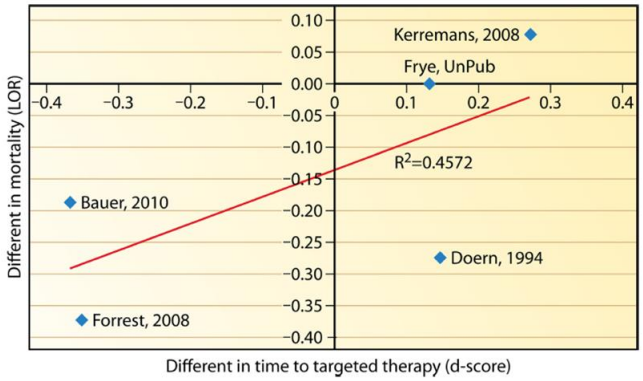
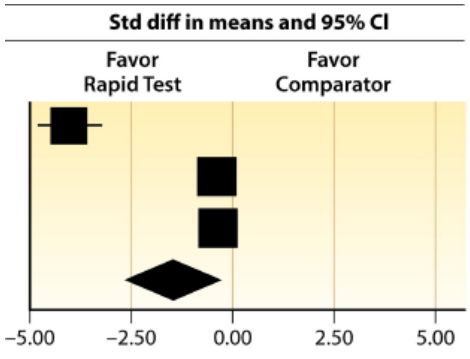
Effectiveness of Improved Timeline of BSI results

Rapid tests & additional communication leads to more timely treatment





Clin Microb Rev 2016; 29: 59-103

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Application of Whole Genome Sequencing

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Next Generation Sequencing (NGS)

1. Identify bacteria via sequence analyses of 16S rDNA & fungi via 18S rDNA
2. Single protocol, as primers needed & different platforms use different sequence technologies
3. Not clear how many alleles 2 genomes may vary to call them close to being or actually identical
4. NGS used for outbreak management, molecular case finding surveillance of pathogens, rapid identification & taxonomy
5. A metagenomic approach can be used to study the resistance

J Biotechnol 2017; 243; 16-24

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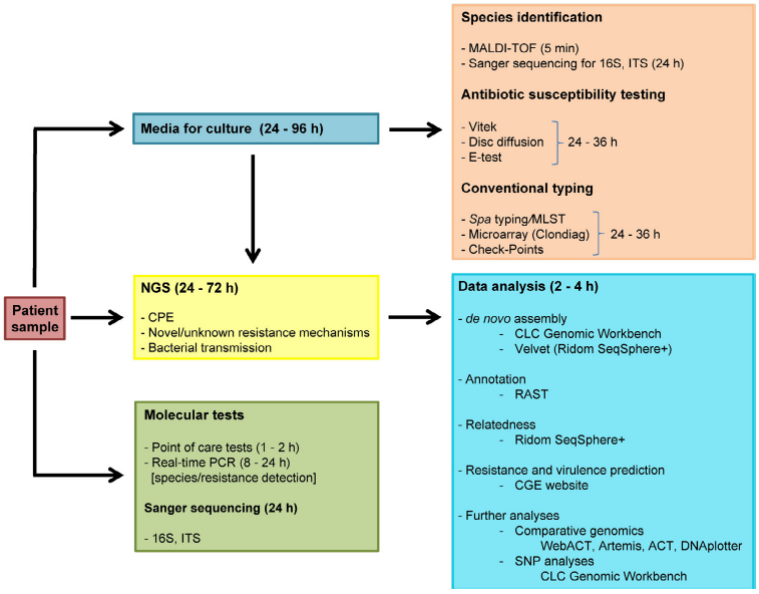
Next Generation Sequencing

Lots of data to analyse, e.g. faeces



J Biotechnnol 2017; 243: 16-24

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16S rDNA & Antimicrobial Stewardship

	Neurosurgical patients (27)	Other patients (33)
16S rDNA detected	18 (53%)	15 (34%)
Antimicrobial details available	18 (87%)	11 (85%)
De-escalation	3 (23%)	3 (18%)

J Hosp Infect (in press)

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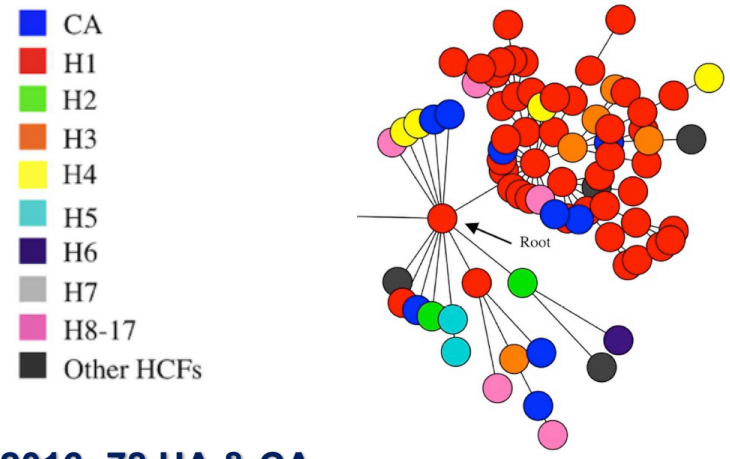
MRSA in Ireland – Hospital & Community



- 89 isolates, June 2013-2016; 78 HA & CA
- Mupirocin-resistant MRSA

PLoS One; 2017: 12(4): e01755432

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Conclusions -1

1. Major changes in microbiology diagnostics
2. Greater accuracy & quicker results
3. Benefits in "downstream" value *vs* "upstream costs"
4. Costs will come down
5. Rationalisation of antibiotic use & earlier information to control outbreaks

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Conclusions-2

6. Technology will drive centralisation & consolidation of laboratories
7. POCT will increase in importance due to consolidation & patient demands
8. The era of culture is not over yet, e.g. urines (cheap & fast enough)
9. Challenges are analysis & interpreting huge amounts of data
10. Clinical need & not availability of technology must drive developments

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The Role of Rapid Diagnostics in Preventing Healthcare Infections
Prof. Hilary Humphreys, Royal College of Surgeons in Ireland
A Webber Training Teleclass

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