Rapid diagnostic solutions for Antimicrobial Resistance

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UK Government report into Anti-Microbial Resistance (O'Neill), 2016

SUMMARY OF RECOMMENDATIONS

5. Promote new, rapid diagnosis

"..support the use of rapid point-of-care diagnosis… to facilitate the mandatory use of tests to support clinical decision making by 2020

Public enemies (WHO 2017)

- **Priority 1: CRITICAL**
- ***Acinetobacter baumannii*, carbapenem-resistant**
- ***Pseudomonas aeruginosa*, carbapenem-resistant**
- ***Enterobacteriaceae*, carbapenem-resistant, ESBL-producing**
- **Priority 2: HIGH**
- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- ***Helicobacter pylori*, clarithromycin-resistant**
- ***Campylobacter* spp., fluoroquinolone-resistant**
- ***Salmonellae*, fluoroquinolone-resistant**

cont..

***Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant**

Priority 3: MEDIUM

Streptococcus pneumoniae, penicillin-non-susceptible

***Haemophilus influenzae*, ampicillin-resistant**

***Shigella* spp., fluoroquinolone-resistant**

Genotypic vs Phenotypic assay

Genotypic (DNA):

Pros:

- Very sensitive (1 copy) and rapid (<30 mins)
- Specific for particular gene targets in particular bacteria

Cons:

- Does the gene mean that bacteria are viable?
- Can miss gene variants (rapid mutations)
- Need separate assays to detect resistance

Phenotypic (Behaviour):

Pros:

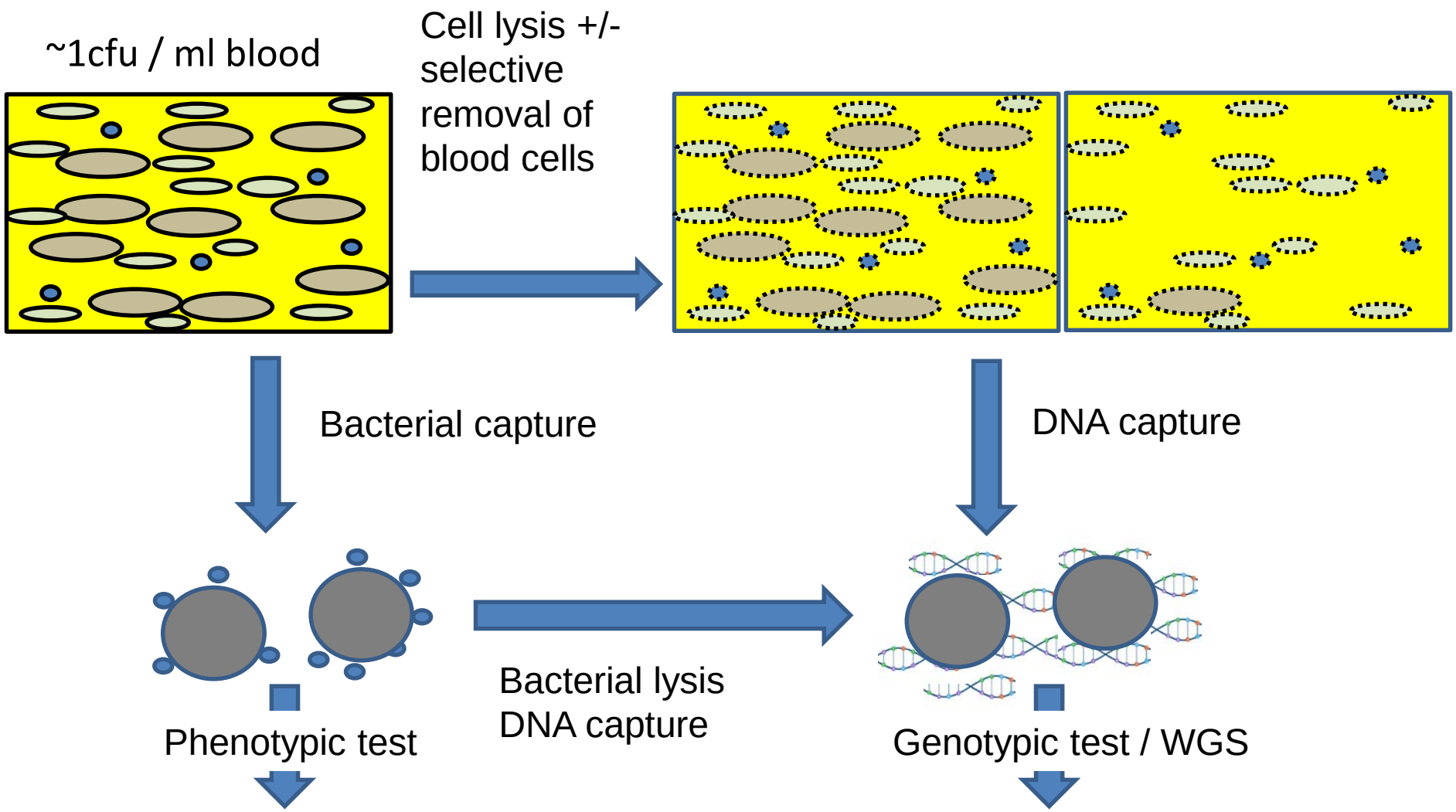
- Measures resistance (enzymes) in viable bacteria.
- Could assess susceptibility to antibiotics

Cons:

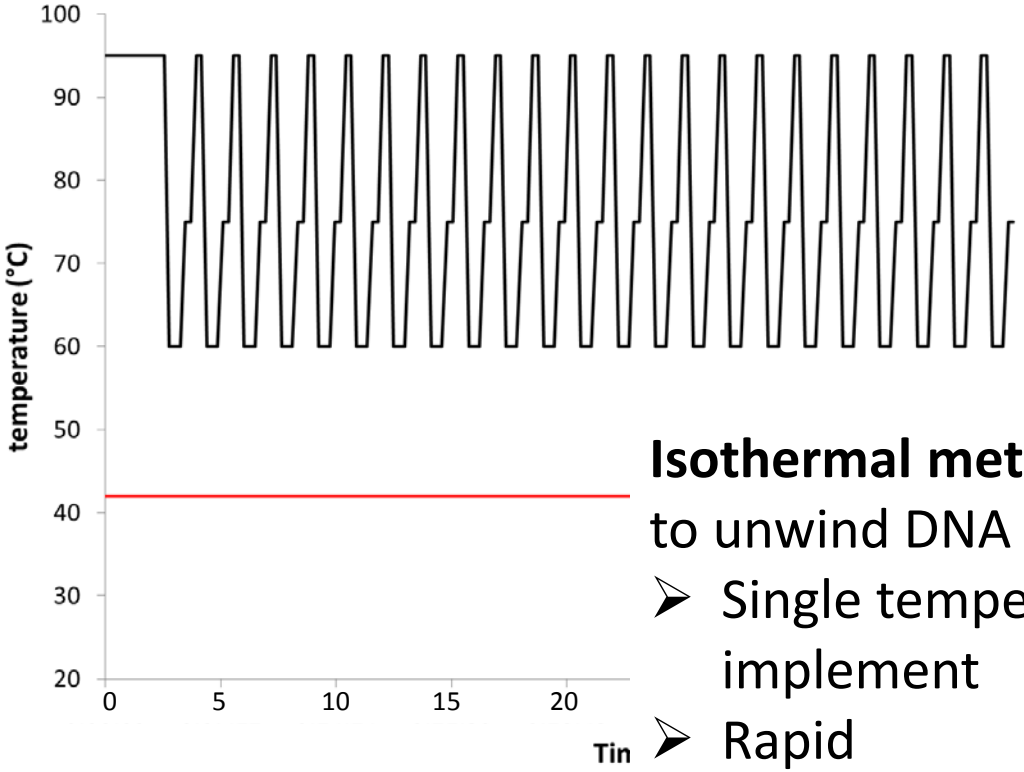
- Low sensitivity - requires culture to amplify (6-8 hours)
- False negatives - samples which do not grow.
- Persister/viable but non-culturable cells not detected

Sample preparation – major issue

Example: Sepsis



Genotypic: Isothermal DNA amplification

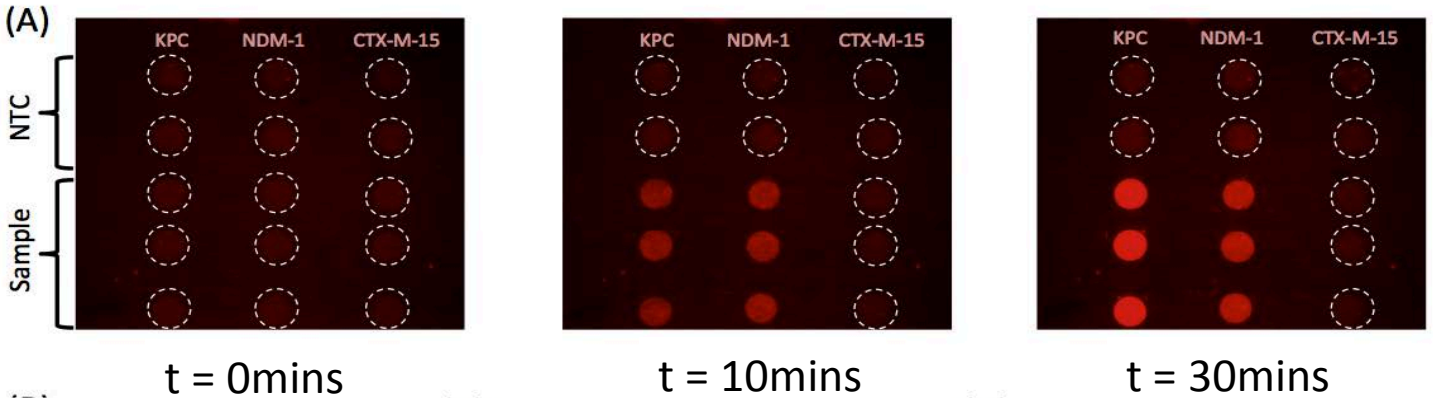


PCR uses temperature cycling to amplify DNA

Isothermal methods use alternative methods to unwind DNA for amplification

- Single temperature; much easier to implement
- Rapid
- Less affected by PCR inhibitors
- Readout with hybridisation fluorescent probe – minimises false positives
- Chemistry v. stable at R.T.

Detection of ESBL/carbapenemase genes on a microfluidic system.

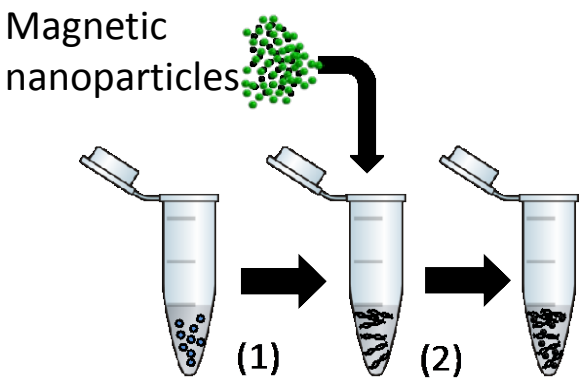


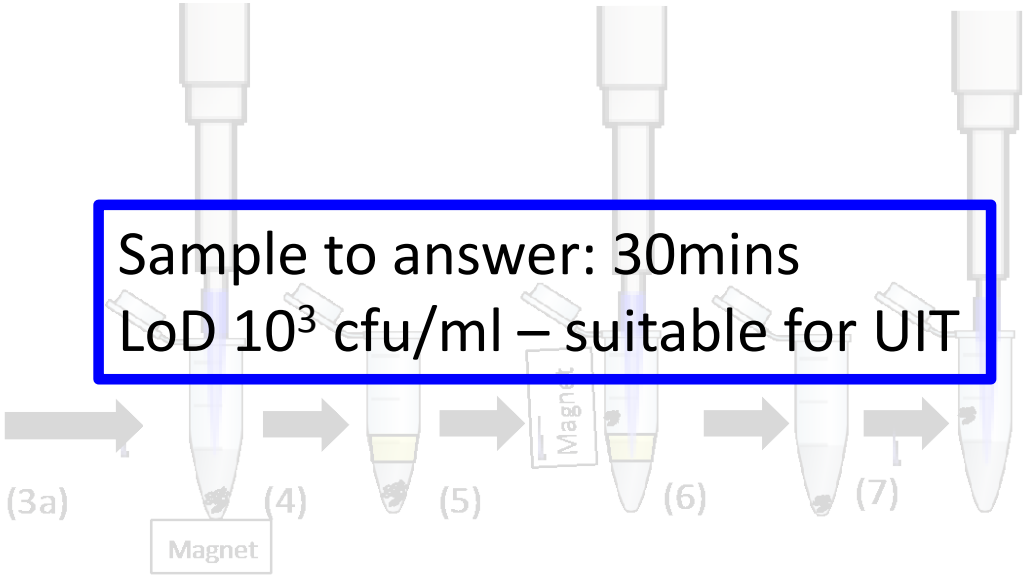
- **Triplex assay**:

  KPC; NDM-1; CTX-M-15 (most prevalent in UK)
- **Simple**: Fluorescence measured with LED/Camera
- **Fast**: 15 mins
- **Sensitive**: LoD of 10 copies
- **Stable**: Assay chemistry stable for weeks at R.T.

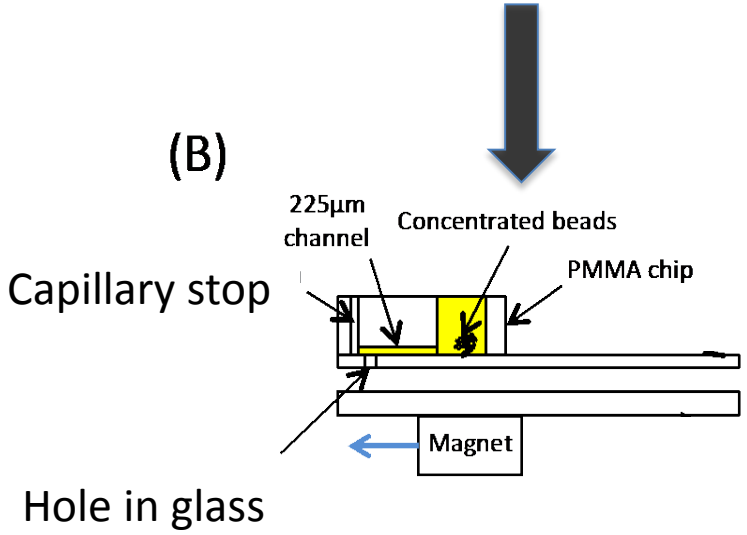
Sample prep (Urinary Tract Infection)

10mL urine
(*E.coli, K. pneumoniae*)





Sample to answer: 30mins
LoD 10^3 cfu/ml – suitable for UIT



10ml ➔ 2.5µL in 2mins

ACCESS

Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by *Escherichia coli* and association with routine use of antibiotics in primary care: systematic review and meta-analysis

Ashley Bryce,[1] Alastair D Hay,[1] Isabel F Lane,[1] Hannah V Thornton,[1] Mandy Wootton,[2] Céire Costelloe[3]

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A Bryce

ABSTRACT

OBJECTIVES

To systematically review studies investigating the prevalence of antibiotic resistance in urinary tract infections caused by *Escherichia coli* in children and, when appropriate, to meta-analyse the relation between previous antibiotics prescribed in primary care and resistance.

DESIGN AND DATA ANALYSIS

Systematic review and meta-analysis. Pooled percentage prevalence of resistance to the most commonly used antibiotics in children in primary care, stratified by the OECD (Organisation for Economic Co-operation and Development) status of the study country. Random effects meta-analysis was used to

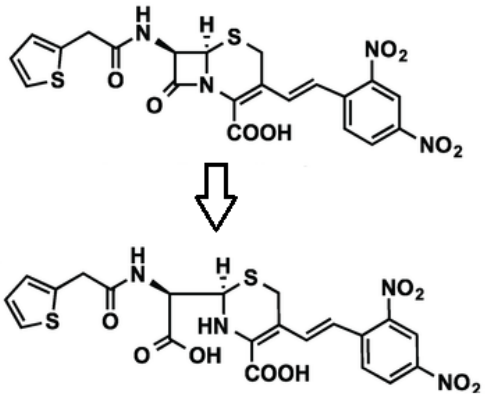
(95% confidence interval 46.0% to 60.8%) for ampicillin, 23.6% (13.9% to 32.3%) for trimethoprim, 8.2% (7.9% to 9.6%) for co-amoxiclav, and 2.1% (0.8 to 4.4%) for ciprofloxacin; nitrofurantoin was the lowest at 1.3% (0.8% to 1.7%). Resistance in studies in countries outside the OECD was significantly higher: 79.8% (73.0% to 87.7%) for ampicillin, 60.3% (40.9% to 79.0%) for co-amoxiclav, 26.8% (11.1% to 43.0%) for ciprofloxacin, and 17.0% (9.8% to 24.2%) for nitrofurantoin. There was evidence that bacterial isolates from the urinary tract from individual children who had received previous prescriptions for antibiotics in primary care were more likely to be resistant to antibiotics, and this increased risk could persist for up to six months (odds ratio 13.23, 95% confidence interval 7.84 to 22.31).

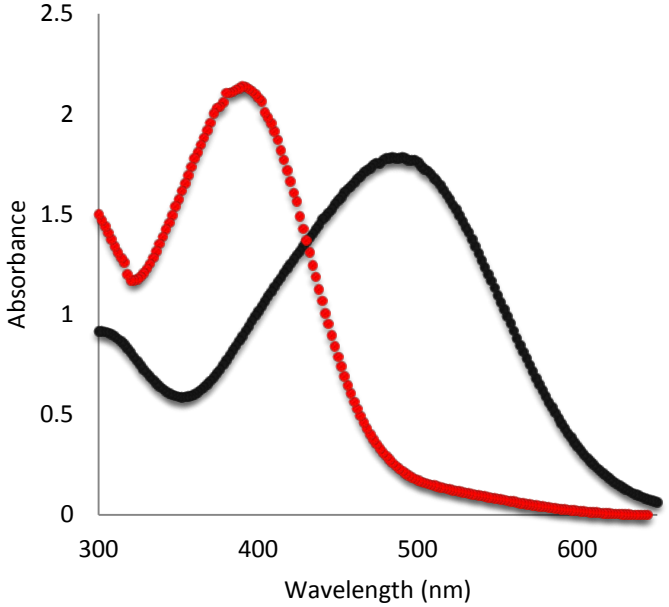
thebmj | *BMJ* 2016;352:i939 | doi: 10.1136/bmj.i939

Phenotypic: Optical

Q: Does the bacteria carry the enzyme?

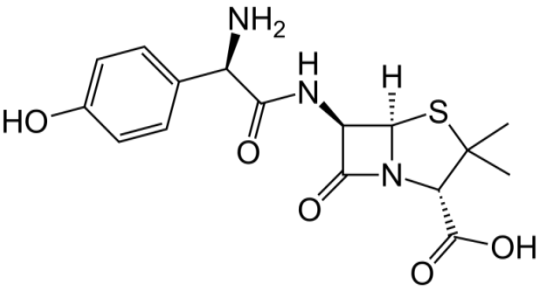
Nitrocefin



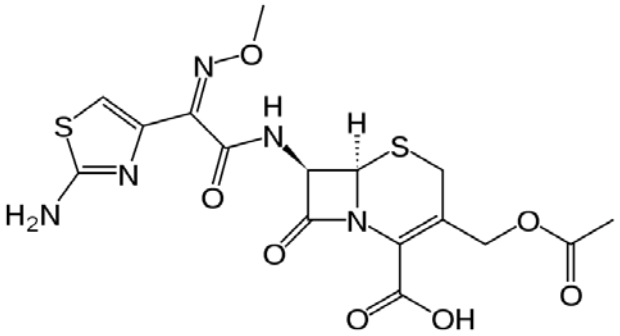


Colour change (red to yellow)

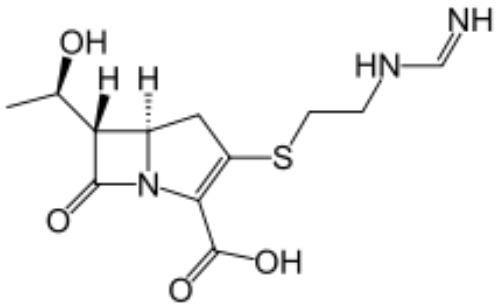
Beta-lactamase

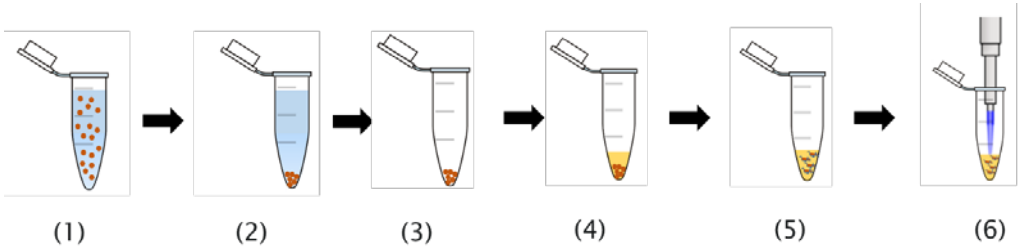


Extended spectrum beta-lactamase (ESBL)

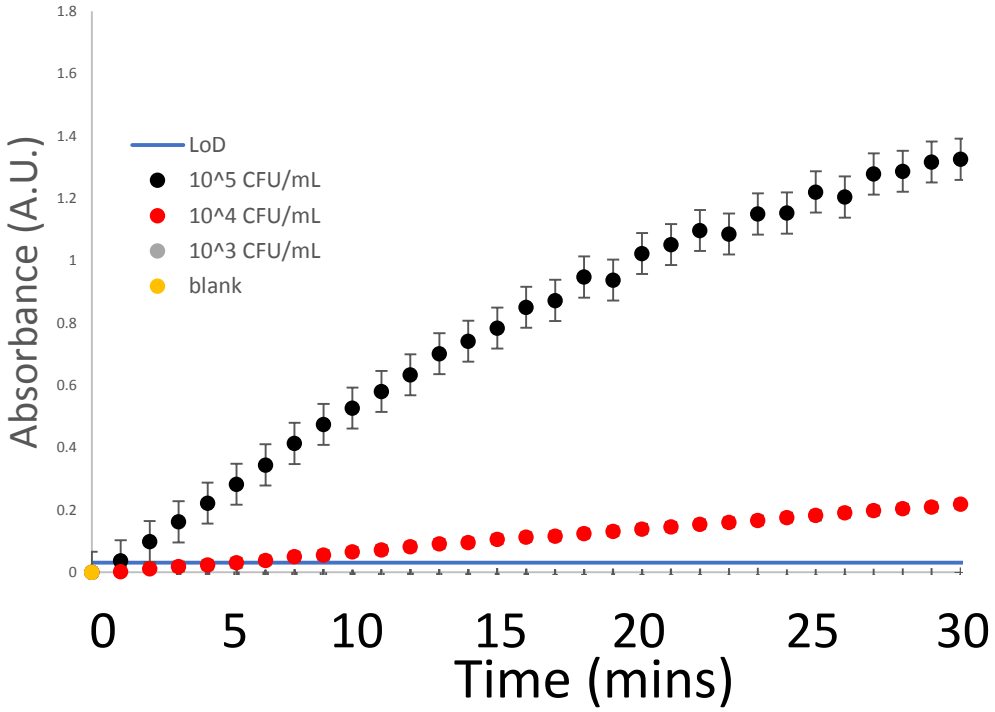


Carbapenemase

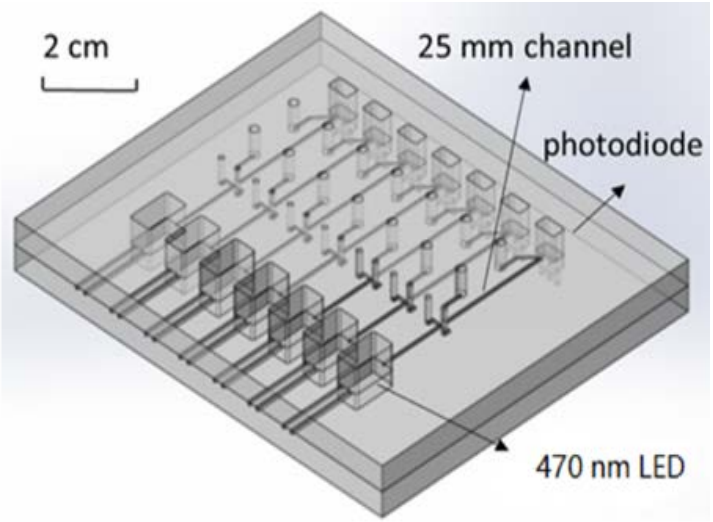


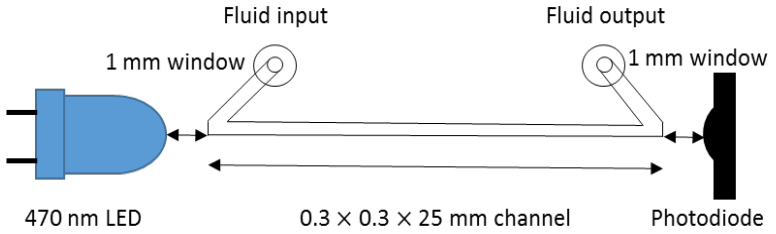


Extract proteins from *E. coli*, mix with nitrocefin, and inject into optical chip.



Multi-channel plastic optical absorption chip





Absorbance change of Nitrocefin for *E. coli* with *bla* $_{\text{CTX-M}}$

Susceptibility

Does the antibiotic kill the bacteria?

Healthy (or resistant) bacteria

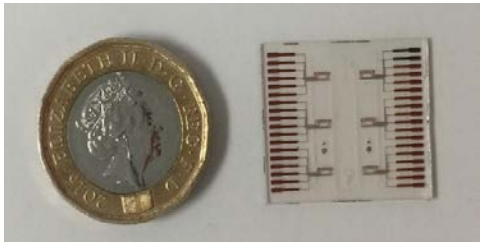
+ antibiotic

Bacteria killed

Signal

No Signal

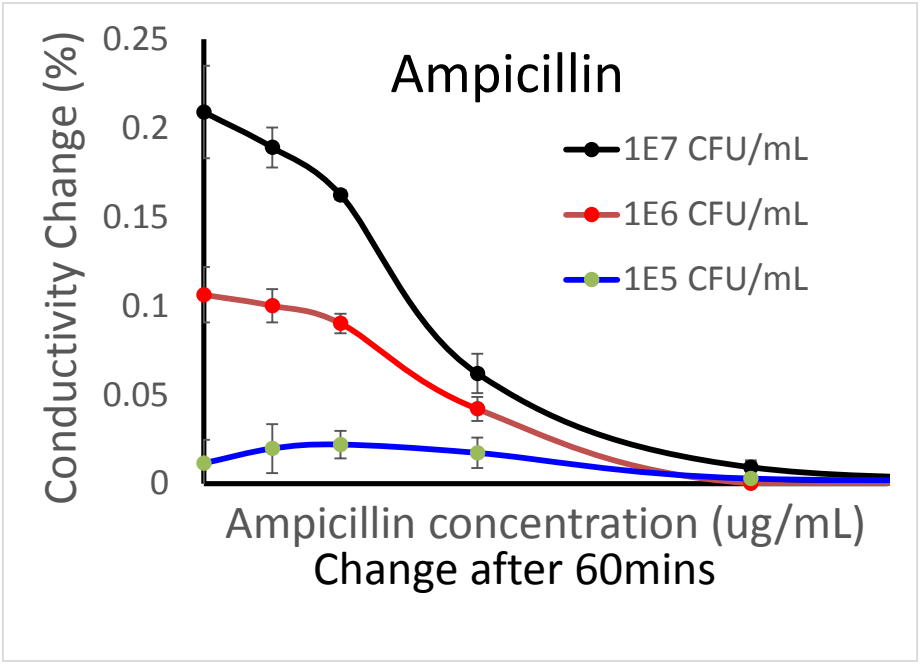
Detect viability



Simple chip < $1

Electrical MIC for *E. coli* ($bla_{CTX\ M15}$)

(Formal MIC = 16μg/mL; 90% kill)



VERY FAST Test in < 1hour

Challenges:

- Fast, simple assays needed for MIC **and** organism I.D.
- Improved sample preparation for isolation of bacteria from clinical matrices, blood, urine (eliminate need to culture);
    - 1-3 cfu/ml blood. Needs to cover wide range of pathogens causing sepsis
- Rare cell isolation and analysis
    - e.g. persistor / tolerant cells possibly driving resistance
- Improve biomarker discovery and validation for infectious disease - different cell types and/or exosomes (hose response).

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DIAGNOSTICS FOR ALL
Low-cost, easy-to-use, point-of-care diagnostics for the developing world.

Nefeli Tsaloglou (CEO)

TwistDx

Olaf Pipenburg
Niall Armes

Technology Strategy Board
Driving Innovation

SHARP

EPSRC
Engineering and Physical Sciences Research Council

THE ROYAL SOCIETY

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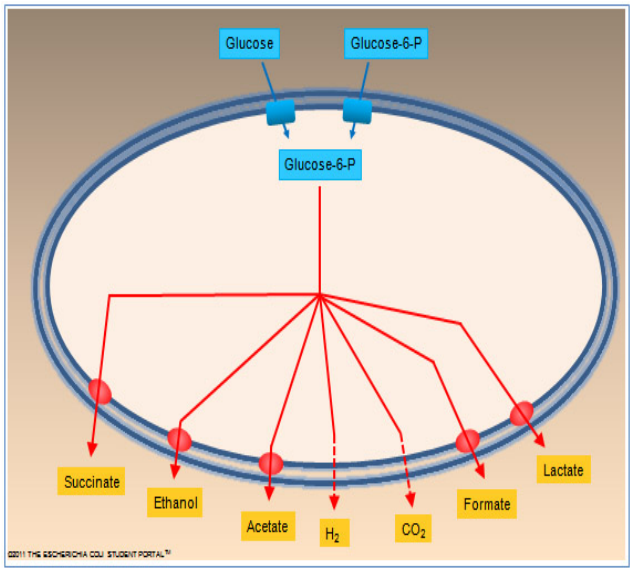
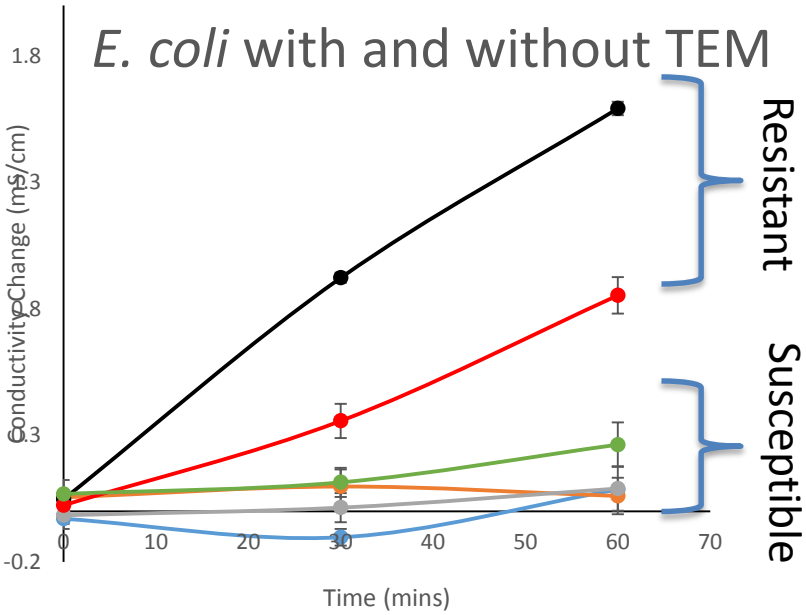
Funded by NHS National Institute for Health Research

Conductivity Sensor



Glucose metabolism in *E. coli.*

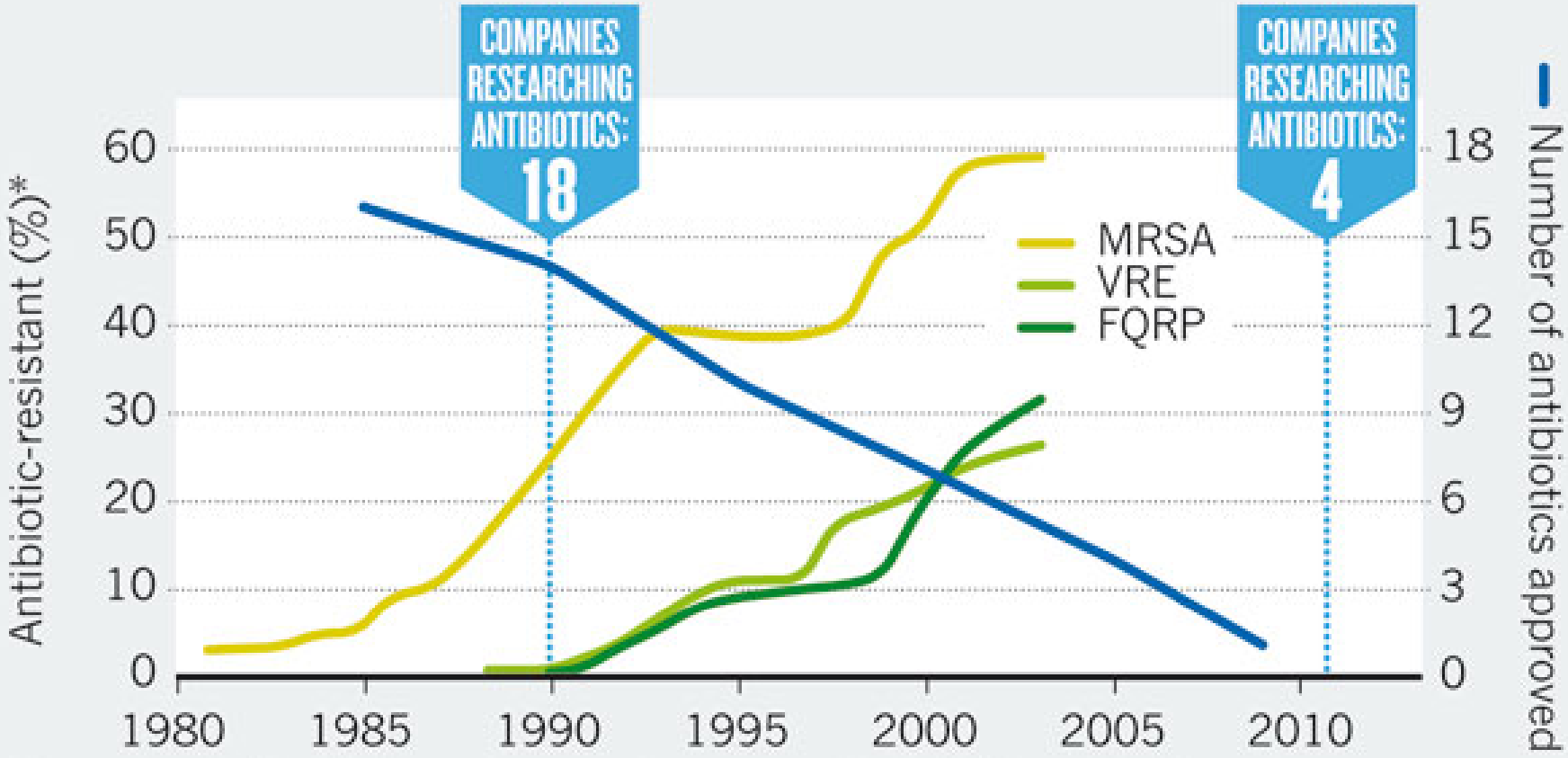


Bacteria metabolise glucose into lactate or carbonate, increasing conductivity of the solution.

"*E. coli* student portal - Fermentation", *Ecolistudentportal.org*, 2018. [Online]. Available: http://ecolistudentportal.org/article_fermentation. [Accessed: 27- Feb- 2018].

A PERFECT STORM

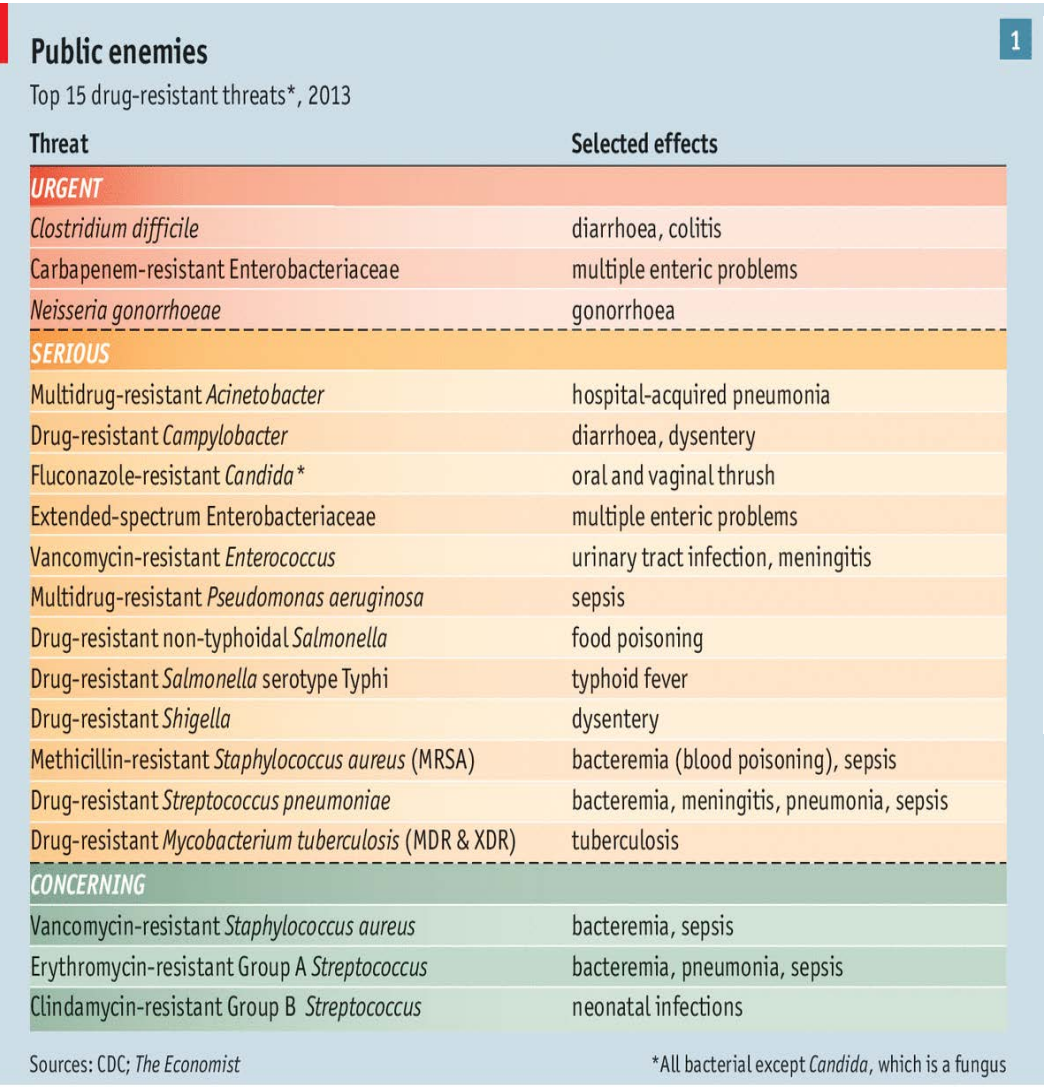
As bacterial infections grow more resistant to antibiotics, companies are pulling out of antibiotics research and fewer new antibiotics are being approved.



*Proportion of clinical isolates that are resistant to antibiotic. MRSA, methicillin-resistant *Staphylococcus aureus*. VRE, vancomycin-resistant *Enterococcus*. FQRP, fluoroquinolone-resistant *Pseudomonas aeruginosa*.

Fix the antibiotics pipeline Matthew A. Cooper[1] David Shlaes[2] Nature 472, p32 (2011)

CDC list 2013 and “ESKAPEE” pathogens



Public enemies 1

Top 15 drug-resistant threats*, 2013

Threat	Selected effects
URGENT	
Clostridium difficile	diarrhoea, colitis
Carbapenem-resistant Enterobacteriaceae	multiple enteric problems
Neisseria gonorrhoeae	gonorrhoea
SERIOUS	
Multidrug-resistant *Acinetobacter*	hospital-acquired pneumonia
Drug-resistant *Campylobacter*	diarrhoea, dysentery
Fluconazole-resistant *Candida**	oral and vaginal thrush
Extended-spectrum Enterobacteriaceae	multiple enteric problems
Vancomycin-resistant *Enterococcus*	urinary tract infection, meningitis
Multidrug-resistant *Pseudomonas aeruginosa*	sepsis
Drug-resistant non-typhoidal *Salmonella*	food poisoning
Drug-resistant *Salmonella* serotype Typhi	typhoid fever
Drug-resistant *Shigella*	dysentery
Methicillin-resistant *Staphylococcus aureus* (MRSA)	bacteremia (blood poisoning), sepsis
Drug-resistant *Streptococcus pneumoniae*	bacteremia, meningitis, pneumonia, sepsis
Drug-resistant *Mycobacterium tuberculosis* (MDR & XDR)	tuberculosis
CONCERNING	
Vancomycin-resistant *Staphylococcus aureus*	bacteremia, sepsis
Erythromycin-resistant Group A *Streptococcus*	bacteremia, pneumonia, sepsis
Clindamycin-resistant Group B *Streptococcus*	neonatal infections

Sources: CDC; *The Economist*

*All bacterial except *Candida*, which is a fungus

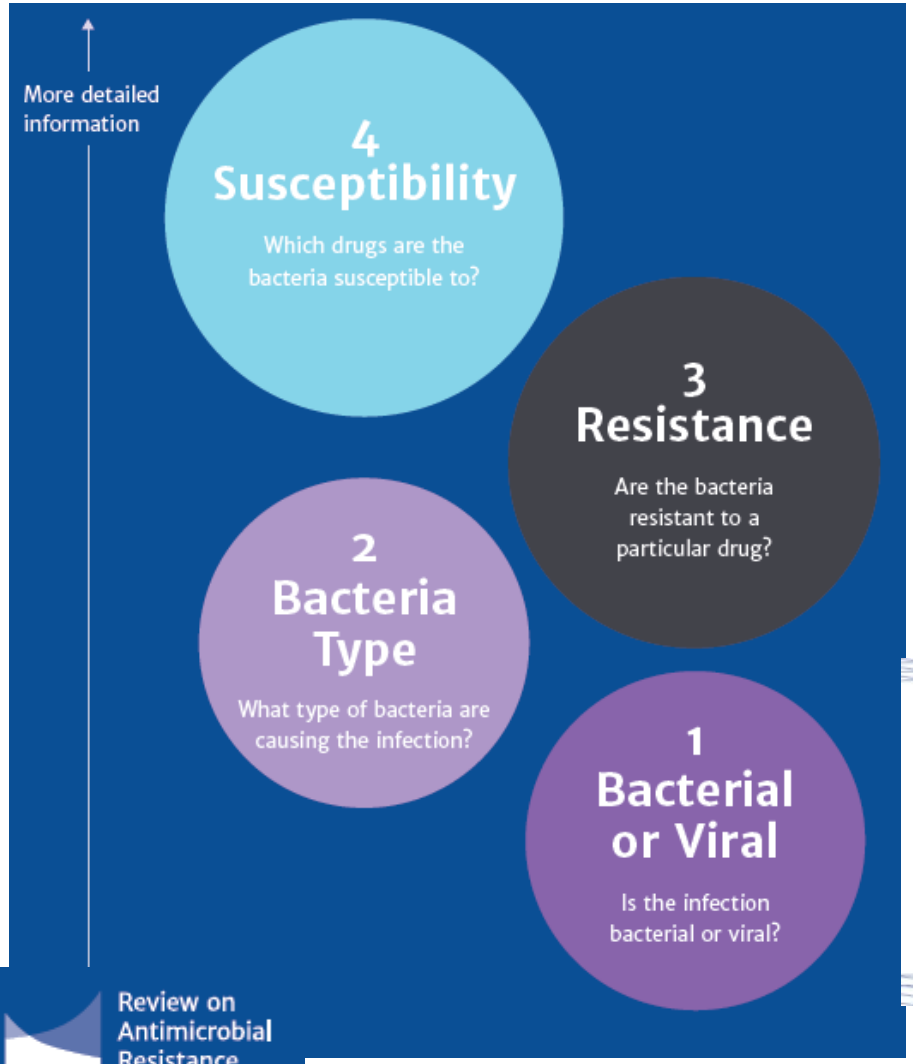
conomist.com

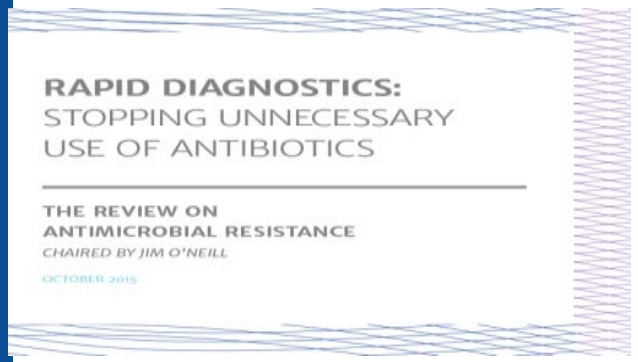
“ESKAPEE” pathogens

E*nterococcus faecalis*
S*taphylococcus aureus*
K*lebsiella pneumoniae* ***A****cinetobacter baumannii* ***P****seudomonas aeruginosa*
E*nterobacter spp*
E*scherichia coli*

And pathogens with intrinsic resistance to antimicrobials *Stenotrophomonas, Proteus, Burkholderia,*

“The perfect new rapid diagnostic test would answer four questions” - AMR Review





The AMR problem

•"Antimicrobial resistance poses a catastrophic threat. If we don't act now, **any one of us could go into hospital in 20 years for minor surgery and die because of an ordinary infection that can't be treated by antibiotics**"

– *Professor Dame Sally Davies. Chief Medical Officer, England. March 2013*

Ultra-rapid detection of resistance genes using semiconductors

Biosensors and Bioelectronics 96 (2017) 281–287

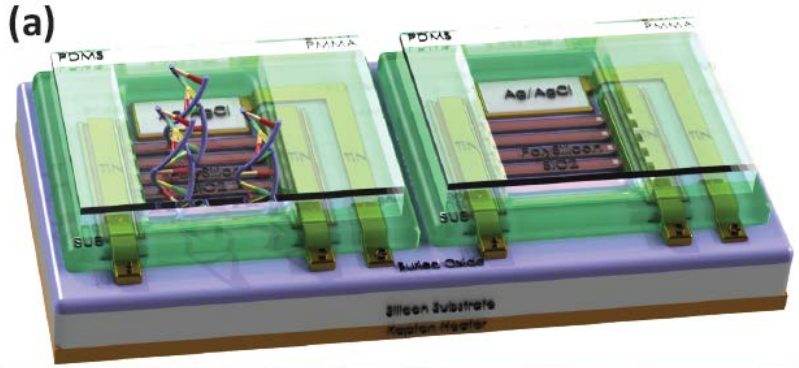
Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

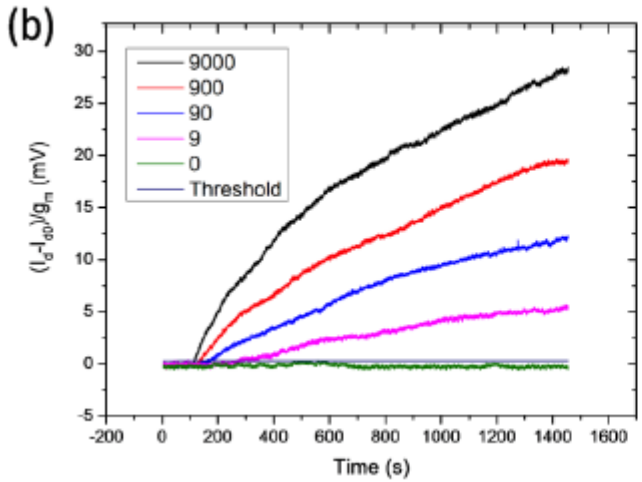
Ultra-fast electronic detection of antimicrobial resistance genes using isothermal amplification and Thin Film Transistor sensors

Chunxiao Hu[a], Sumit Kalsi[a], Ioannis Zeimpekis[a], Kai Sun[a], Peter Ashburn[a], Carrie Turner[b], J. Mark Sutton[b], Hywel Morgan[a,*]

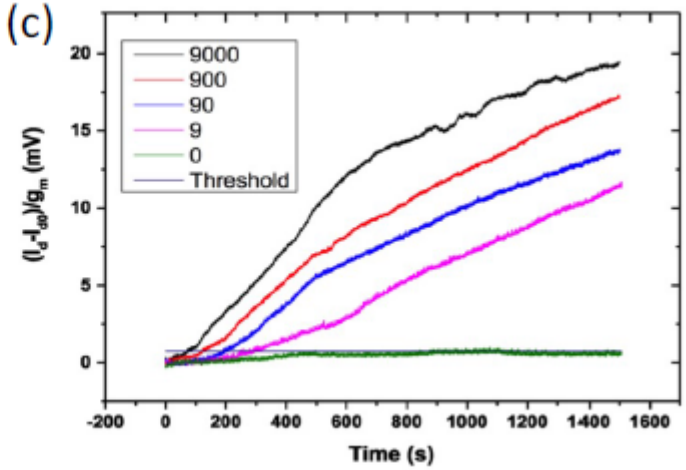


10 copies in 5 mins

CTX-M



KPC



WHO antibiotic resistance quiz

http://www.who.int/campaigns/world-antibiotic-awareness-week/quiz/en/

- **Quiz: How much do you know about antibiotic resistance?**
- **1 Antibiotics are powerful medicines that help to fight:**
- Viruses Bacteria All microbes
- **2 Antibiotic resistance happens when my body becomes resistant to antibiotics.**
- True False
- **3 Antibiotic-resistant bacteria can spread to humans through:**
- Contact with a person who has an antibiotic-resistant infection
- Contact with something that has been touched by a person who has an antibiotic-resistant infection (e.g. a health-workers' hands or instruments in a health facility with poor hygiene)
- Contact with a live animal, food or water carrying antibiotic-resistant bacteria.
- All of the above
- **4 What can happen if I get an antibiotic-resistant infection?**
- I may be sick for longer
- I may have to visit my doctor more or be treated in hospital
- I may need more expensive medicine that may cause side effects
- All of the above
- **5 Antibiotic resistance is already out of control and it's only getting worse. There's nothing I can do.**
- True
- False
- **6 I can help tackle antibiotic resistance if I:**
- Share my antibiotics with my family when they are sick
- Get antibiotics as soon as I feel sick - either directly from the pharmacy or a friend
- Keep my vaccinations up to date

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- True False
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