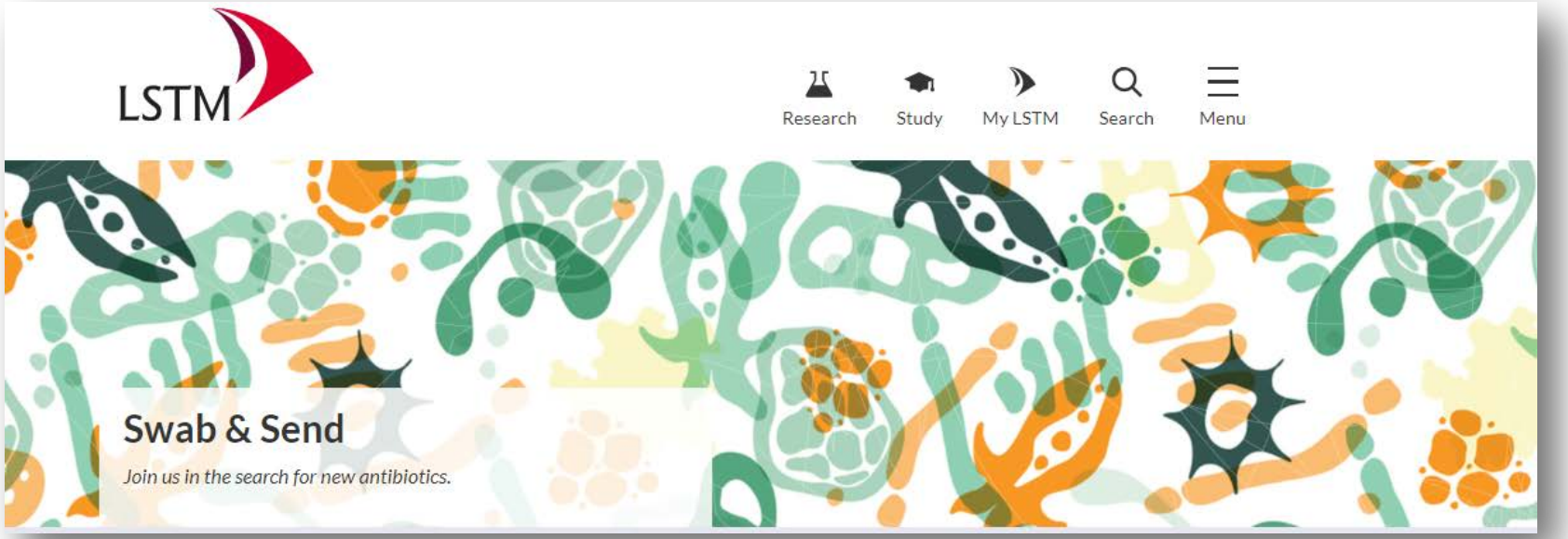
LSTM
LIVERPOOL SCHOOL
OF TROPICAL MEDICINE

Swab and Send – discovering new antimicrobials from natural products

Dr Adam Roberts
@GCAGATGCAATG
#swabandsend

Introduction



Citizen-science
Crowd-funded economic model for early drug discovery
It is portable
Open access results and resources, everything is fed back to participants
Feeds into traditional drug development pipelines and scientific enquiry suitable for further funding

New drugs and public engagement

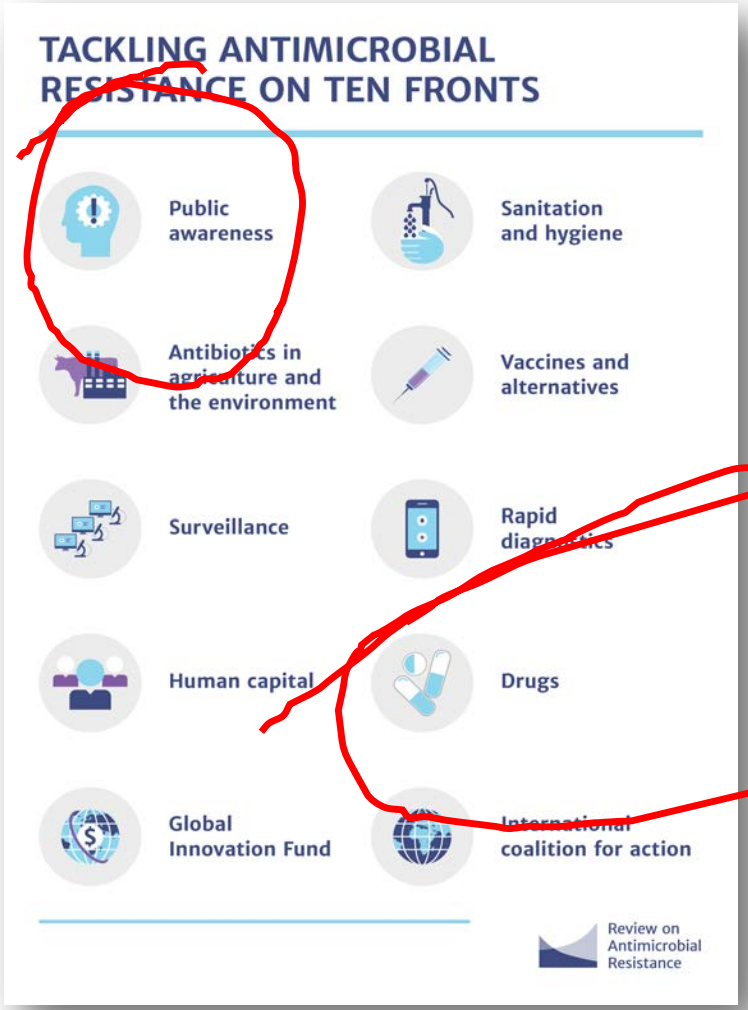
- Increased awareness of AMR and the issues surrounding the difficulties of finding new antibiotics

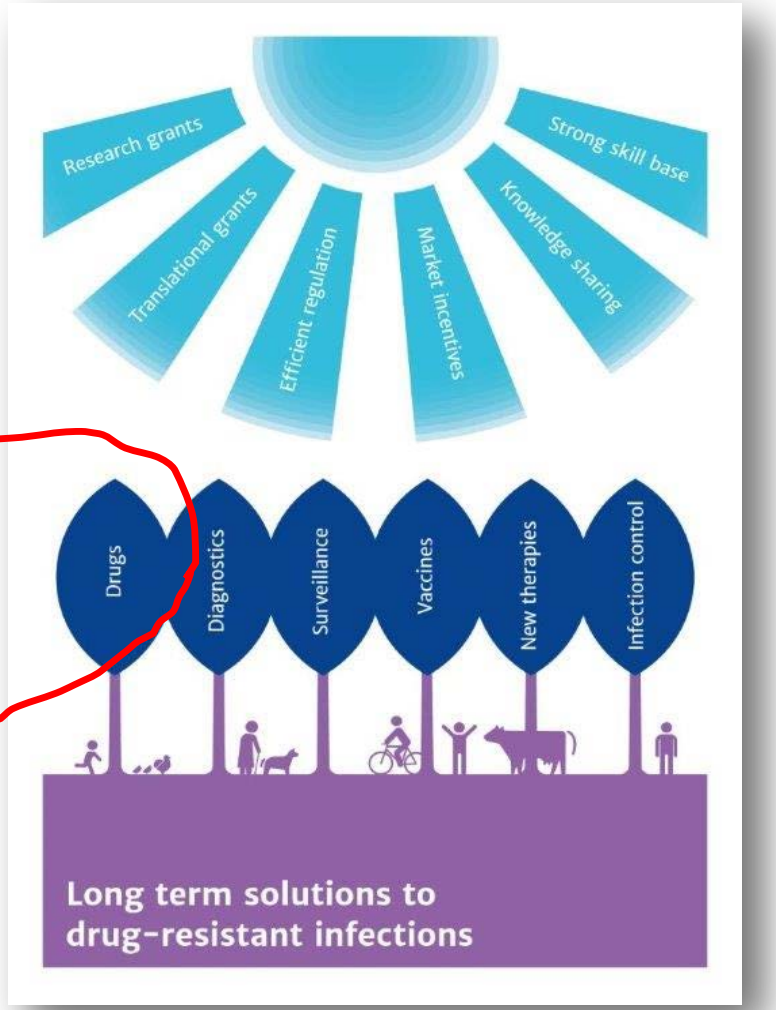
- Why?
- Societal understanding of their value and behavior change is one of multiple prerequisites for solution

What did I want to achieve?

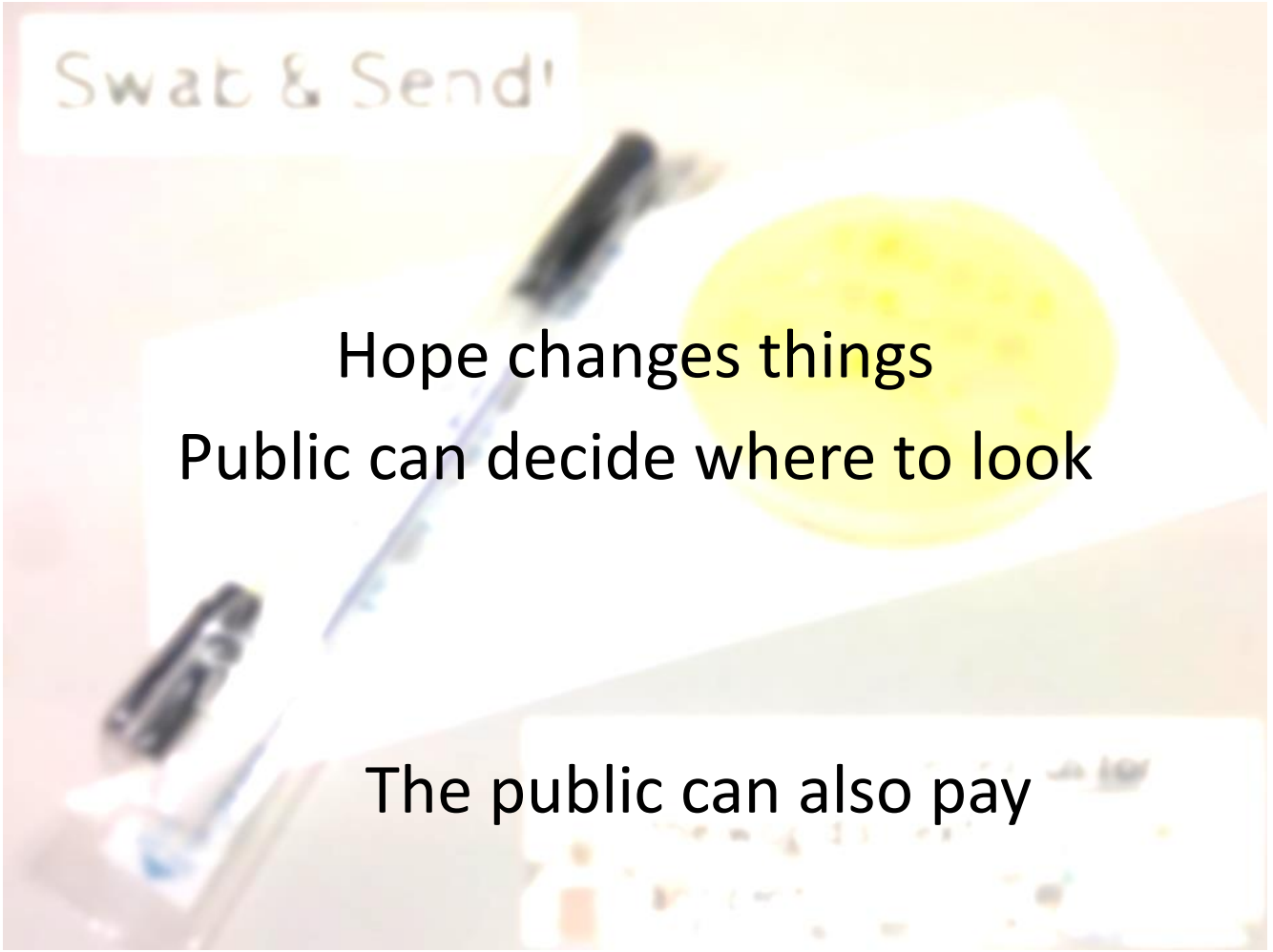
- A happier story than normal (with respect to AMR)

What did I want to achieve?



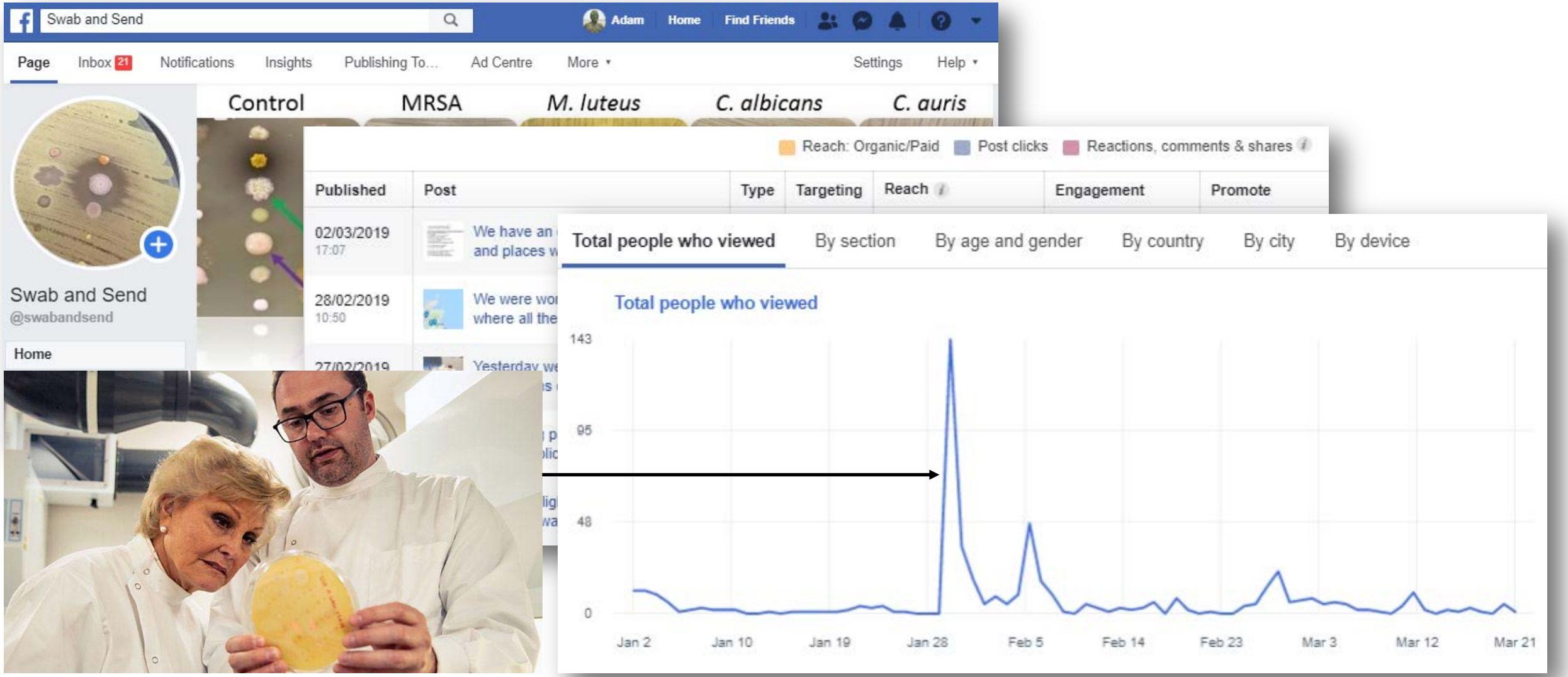


Swab and Send



- Individuals choose were or what to swab.
- Pledge small amount
- Receive Swabs
- Send them back
- Swabs received from all over the UK, from Iceland, Sweden, Japan, Germany, Egypt, Canada, USA.
- We assay individual isolates for antimicrobial production
- Post results onto the project website

Web platform



Early History

- Launched Feb 2015 using Hubbub crow
- First Swabs received May 2015
- July 2015 Requests for school & college

Trust me i'm a Doctor!

6,099,777 Views

BBC Scotland
20 January 2016 ·

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The world has a problem with antibiotic resistance - beards could hold the answer.

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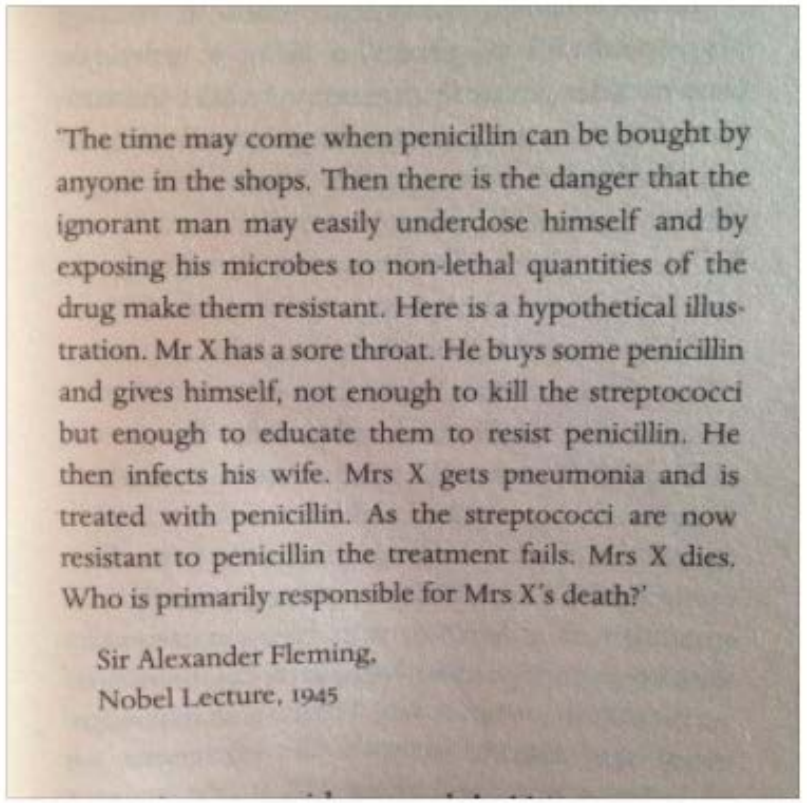
Post Details

Swab and Send
Published by Adam Roberts [?] · 1 January 2016 ·

From a time long long ago. Worth a quick read followed by a long discussion.



'The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant. Here is a hypothetical illustration. Mr X has a sore throat. He buys some penicillin and gives himself, not enough to kill the streptococci but enough to educate them to resist penicillin. He then infects his wife. Mrs X gets pneumonia and is treated with penicillin. As the streptococci are now resistant to penicillin the treatment fails. Mrs X dies. Who is primarily responsible for Mrs X's death?'

Sir Alexander Fleming,
Nobel Lecture, 1945

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5.1k 177 Comments 11k Shares

Performance for your post

2,131,165 People Reached

66,758 Reactions, comments & shares

49,901 Like	5,114 On post	44,787 On shares
18 Love	2 On post	16 On shares
4 Haha	1 On post	3 On shares
16 Wow	0 On post	16 On shares
4 Sad	0 On post	4 On shares
4,988 Comments	301 On Post	4,687 On Shares
11,829 Shares	11,824 On Post	5 On Shares

103,155 Post Clicks

43,841 Photo views	143 Link clicks	59,171 Other Clicks

NEGATIVE FEEDBACK

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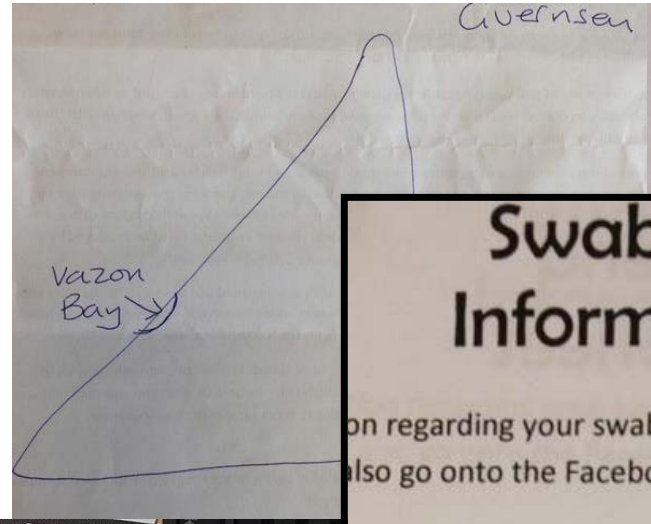
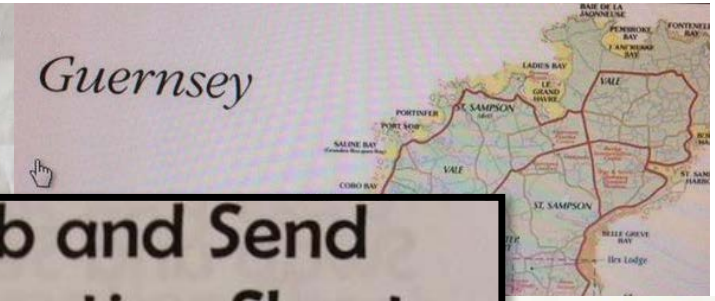
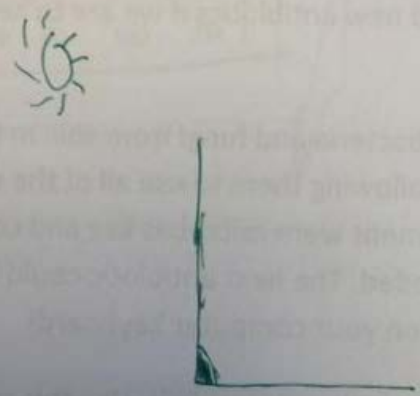
What has been sampled?

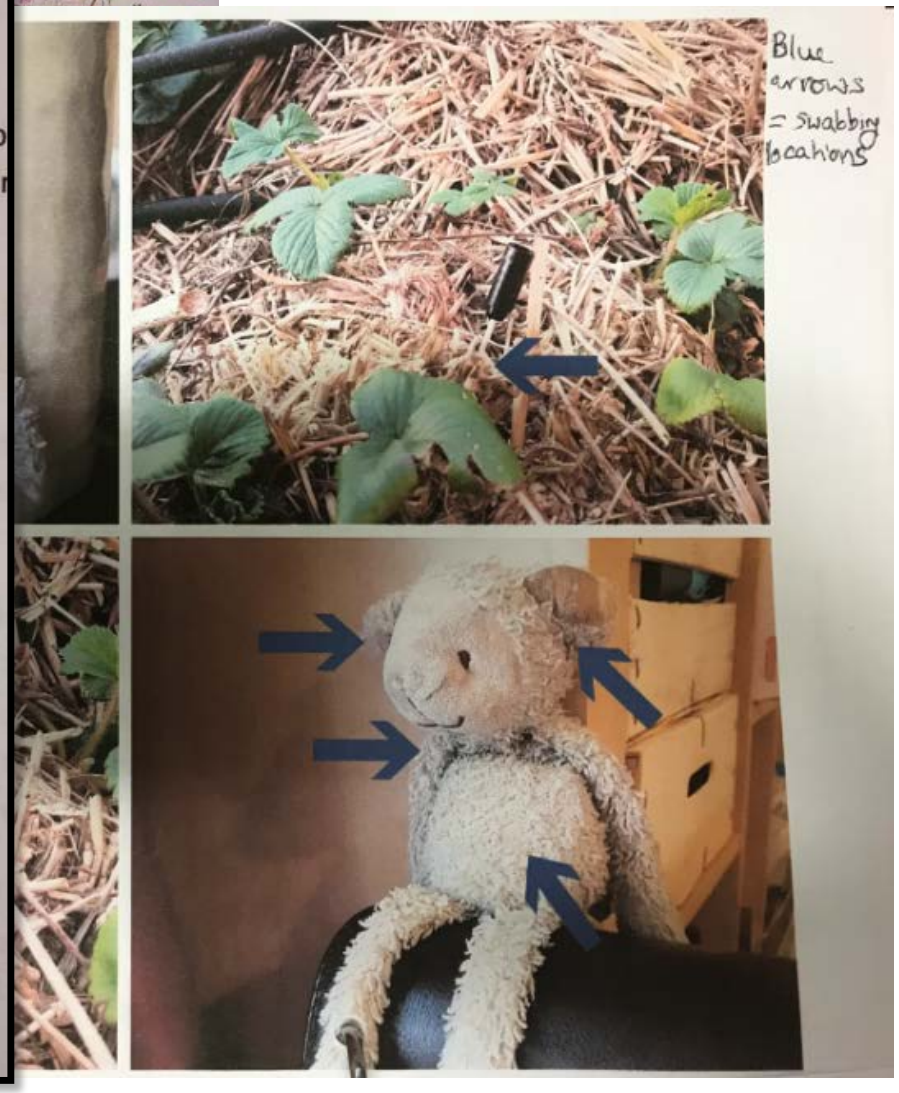




Swab and Send Information Sheet

on regarding your swab or the place that has been swabb
also go onto the Facebook page, whilst primarily for childr





BBC Dalek may yield new antibiotic, 09/06/2016, BBC Inside Science - BBC Radio 4

Our Doctor (Rutherford) has been searching for novel antibiotics in Broadcasting House.

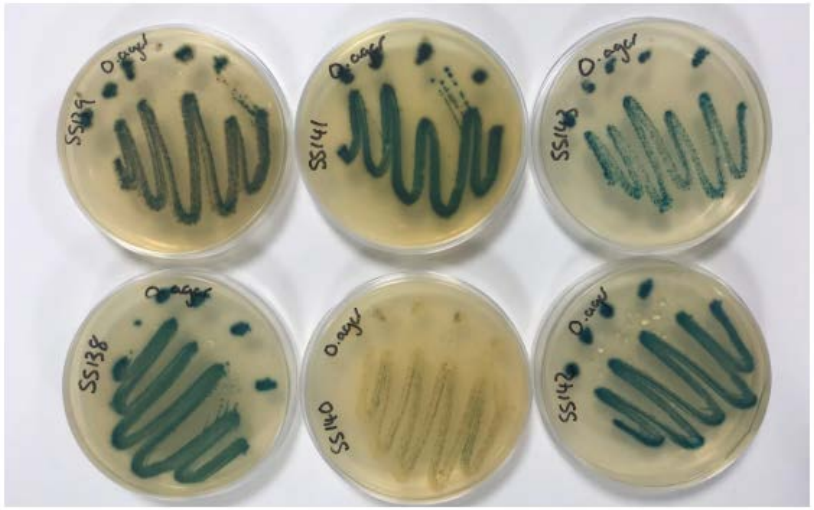
BBC.CO.UK

Is your office water bottle a health hazard? We swabbed ours to find out

Klebsiella killed the cat CREDIT: GEOFF PUGH

Follow
By **Tom Ough**
14 MAY 2018 • 11:00AM

Slowly, slowly catchy E. coli CREDIT: GEOFF PUGH



Burn all the bottles. Burn them all CREDIT: HANDOUT

"I would not drink from any of those bottles," he told me. Ouch. We'd avoided the pink agar jelly bloom of E. coli, but five dishes out of six (shout-out to Olivia, the only non-horrid member of our team) had dark green smears across them. "It's probably going to be Klebsiella,"

Spare me the bacteria paranoia – what doesn't kill us makes us stronger

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BOUDICCA FOX-LEONARD

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INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Passet and Brisse, *Int J Syst Evol Microbiol* 2018;68:
DOI 10.1099/ijsem.0.002517

Description of *Klebsiella grimontii* sp. nov.

Virginie Passet and Sylvain Brisse*

Abstract

Strains previously identified as *Klebsiella oxytoca* phylogroup Ko6 were characteri
genome-sequence based average nucleotide identity analysis and their bi
sequencing demonstrated that the Ko6 strains formed a well-demarcated seq
Klebsiella oxytoca (which includes strains previously labelled as *K. oxytoca* ph
(Ko1). The average nucleotide identity values of Ko6 with *K. oxytoca* and *K. michiga*
The inability to metabolize melezitose differentiated most of the Ko6 strains fron
its genetic and phenotypic characteristics, we propose the name *Klebsiella grimontii* for the Ko6 sequence cluster, with strain 06D021^T (=CIP111401^T, DSM 105630^T) as the type strain. Strains of *Klebsiella grimontii* were isolated from human blood cultures, wound infections, antibiotic-associated haemorrhagic colitis and faecal carriage.

JBC ARTICLE

A tricyclic pyrrolobenzodiazepine produced by *Klebsiella oxytoca* is associated with cytotoxicity in antibiotic-associated hemorrhagic colitis

Received for publication, April 18, 2017, and in revised form, September 19, 2017 Published, Papers in Press, September 26, 2017, DOI 10.1074/jbc.M117.791558

Herman Tse[‡§¶‖], Qiangshuai Gu[**], Kong-Hung Sze[‡§¶‖], Ivan K. Chu[**], Richard Y.-T. Kao[‡§¶‖], Kam-Chung Lee[‡§¶‖], Ching-Wan Lam[‡‡], Dan Yang[**], Sherlock Shing-Chiu Tai[**], Yihong Ke[‡], Elaine Chan[‡], Wan-Mui Chan[‡], Jun Dai[‡], Sze-Pui Leung[‡], Suet-Yi Leung[‡‡], and Kwok-Yung Yuen[‡§¶‖1]

From the [‡]Department of Microbiology, [§]Research Centre of Infection and Immunity, and the [‖]Carol Yu Centre for Infection, Departments of [**]Chemistry and [‡‡]Pathology, the University of Hong Kong and the [¶]State Key Laboratory of Emerging Infectious Diseases, Hong Kong SAR, Hong Kong, China

Edited by Chris Whitfield

kleboxymycin

frontiers in Microbiology

ORIGINAL RESEARCH
published: 11 September 2018
doi: 10.3389/fmicb.2018.02170

Klebsiella grimontii, a New Species Acquired Carbapenem Resistance

Lu Liu[1,2†], Yu Feng[1,2†], Yiyi Hu[1,2], Mei Kang[3], Yi Xie[3] and Zhiyong Zong[1,2,4,5]*

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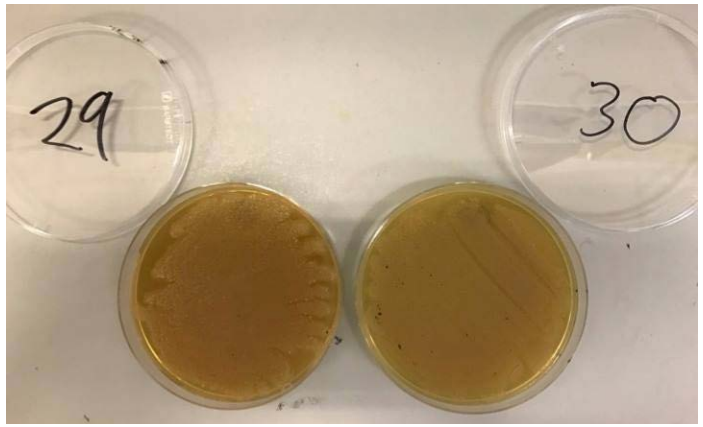
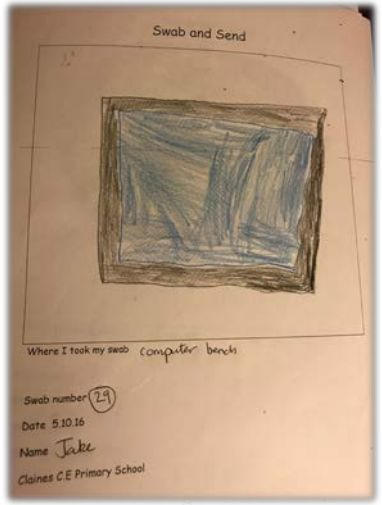
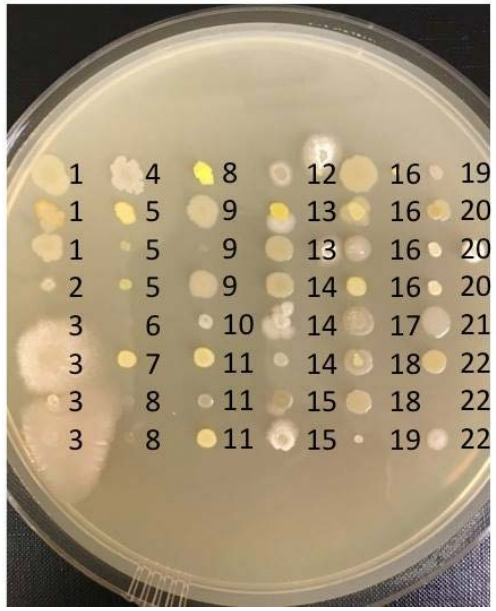
Klebsiella grimontii is a newly identified species closely related to *Klebsiella oxytoca*, but carbapenem resistance was not identified in the species before. We found a carbapenem-resistant *K. oxytoca*-like clinical strain, WCHKG020121. The strain was

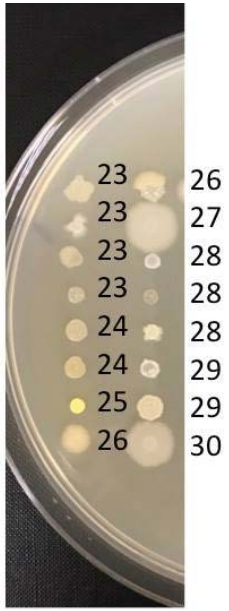
ampicillin (AMP), amoxicillin (AMX), amoxicillin-clavulanic acid (AMC), ceftriaxone (CEF), ciprofloxacin (CIP), olaquindox (OLA), chloramphenicol (CHL) and fosfomycin (FOF)

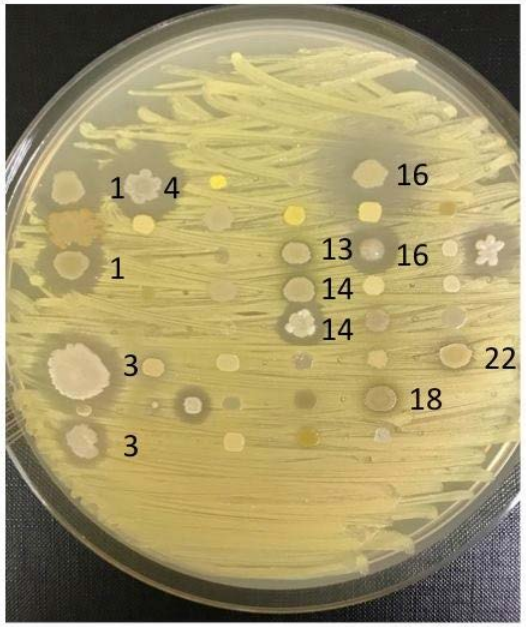
From a computer bench at Claines CE Primary School, Worcestershire.

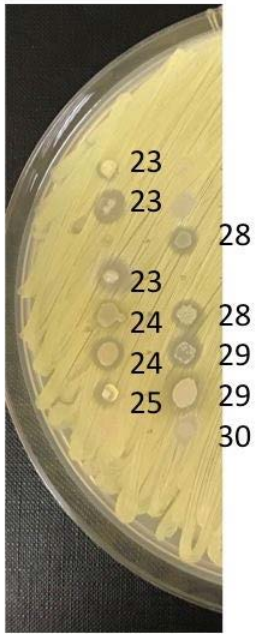




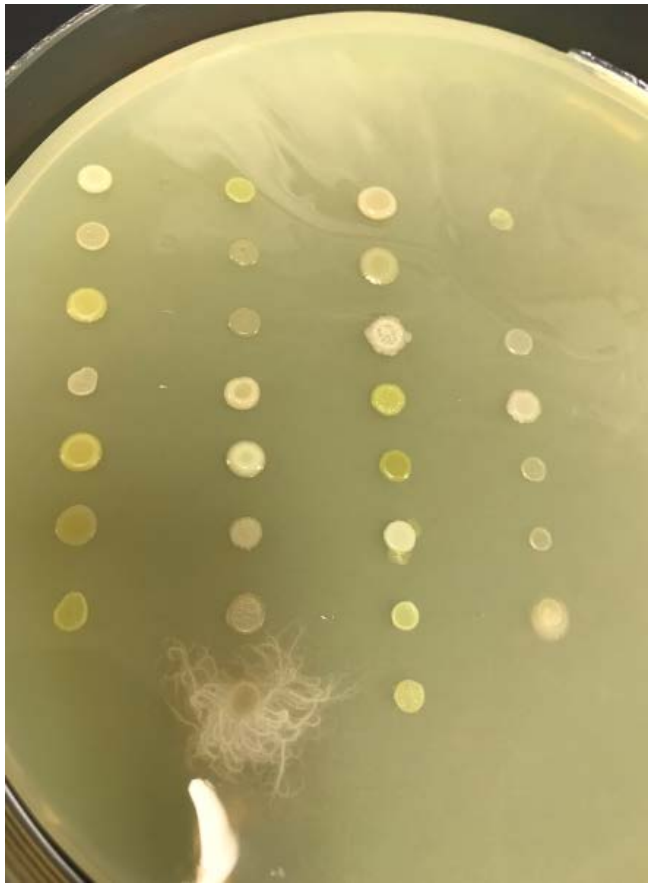




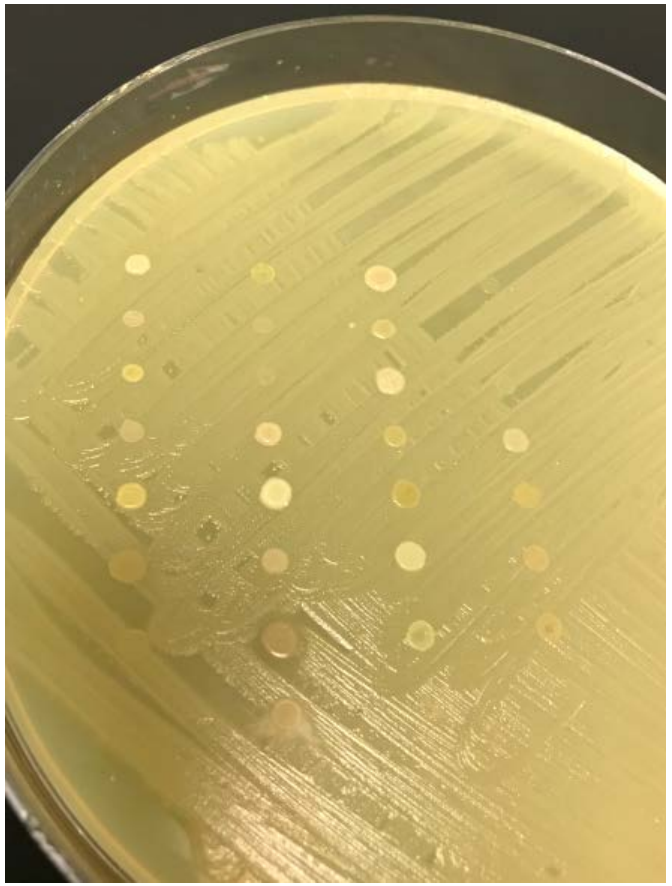




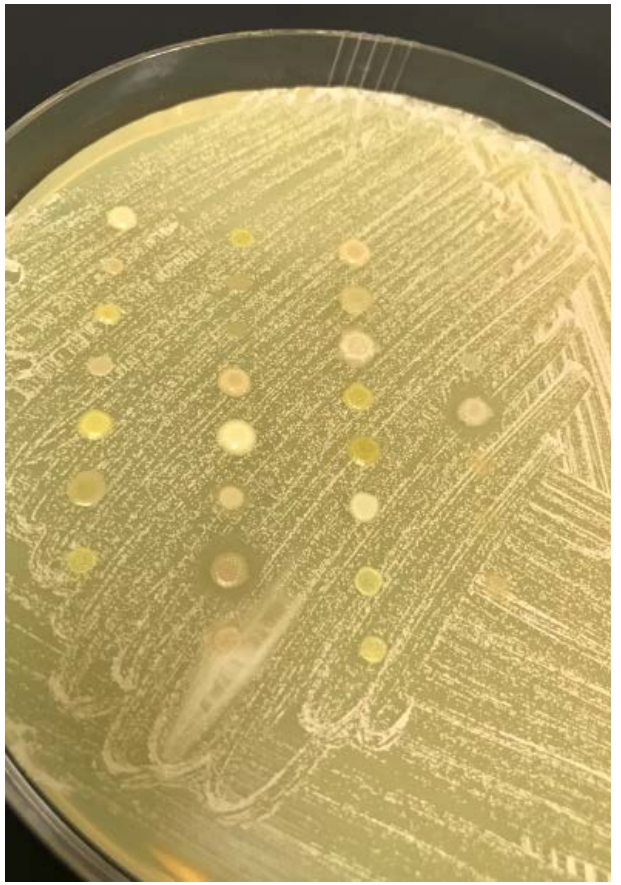
No indicator strain



Multi-drug resistant *Escherichia coli*



Candida albicans



Results to date

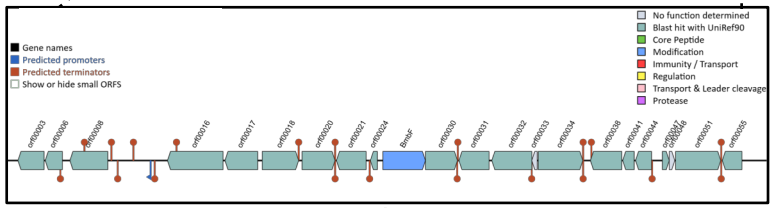
1000s swabs received so far
£1000s funds generated to support the analysis and sequencing
Many thousands of isolates purified and stored assay ready in glycerol stocks in microtitre plates
High 100s inhibit *Micrococcus luteus*
approx. 100 inhibit MRSA
~50 inhibit MDR *Escherichia coli*
~30 inhibit *Candida albicans*
~30 inhibit *Candida auris*
Approx. 50 isolates sequenced so far;
E. coli and / or Candida spp. inhibitors

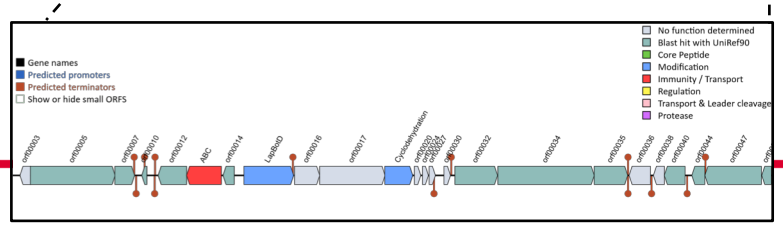
Isolate	Source	*C. albicans*	*C. auris*	*E. coli*	*M. luteus*	*S. aureus*
SS300	Mobile phone		x			
SS222	Desk drawer	x	x		x	
SS211	Dirt	x	x			
SS210	Mud	x	x	x	x	x
SS209	Moss	x	x			
SS171	Magnifying glass		x	x	x	x
SS154	Plug socket	x	x			
SS141	Coffee cup	x	x		x	
SS120	Handkerchief		x		x	x
SS116	Leaf mould	x				

Multiple BGCs can be found

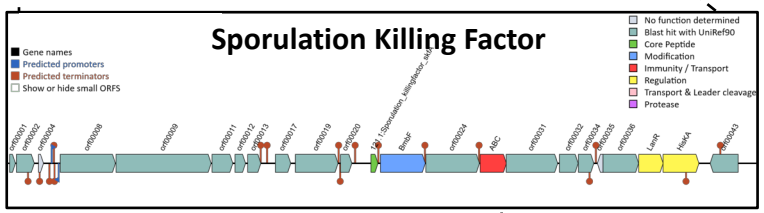
Bacillus sp.

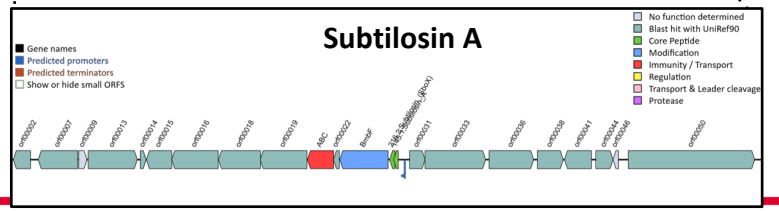
Two uncharacterised BGC





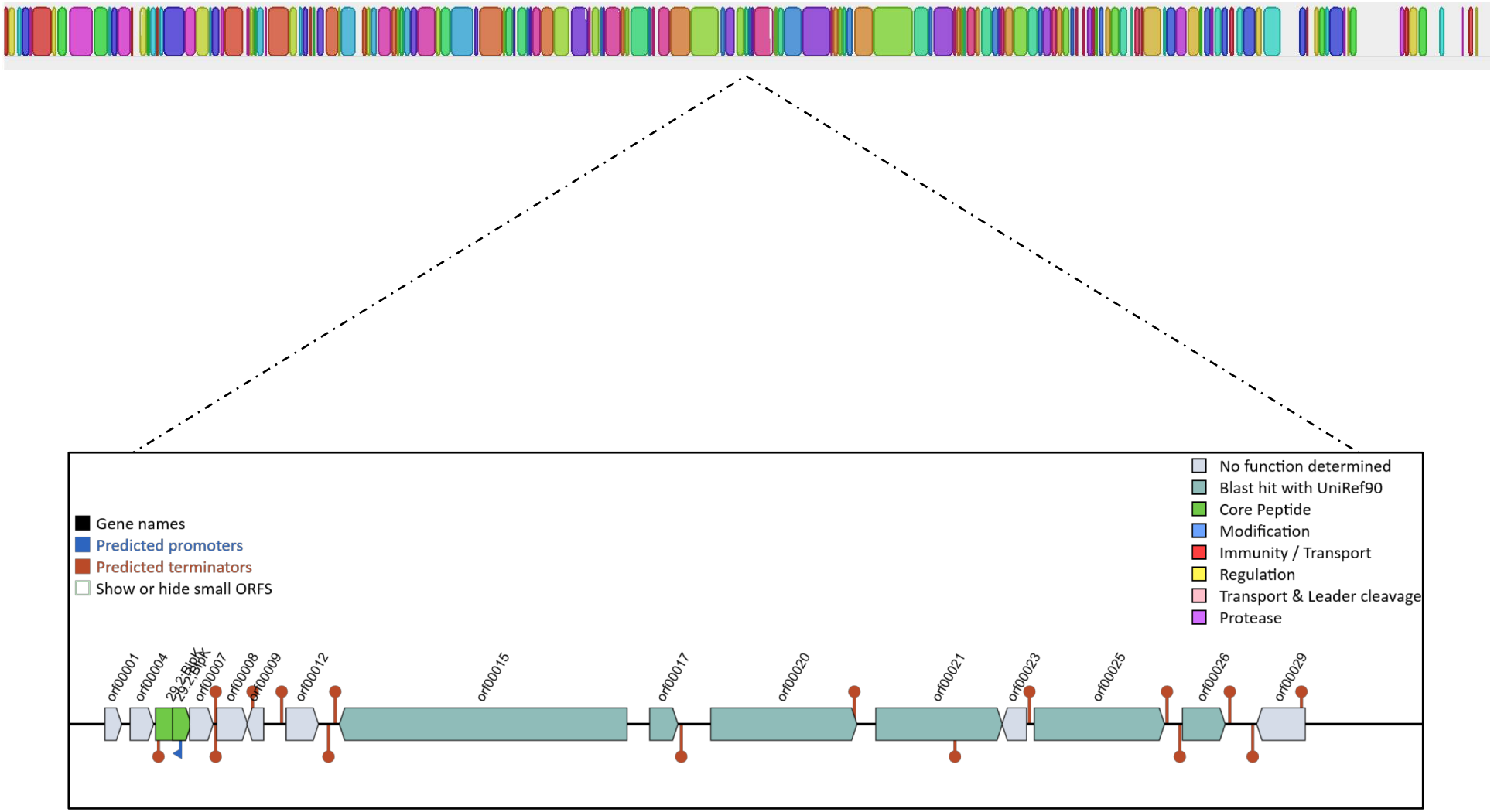
Two previously characterised BGC





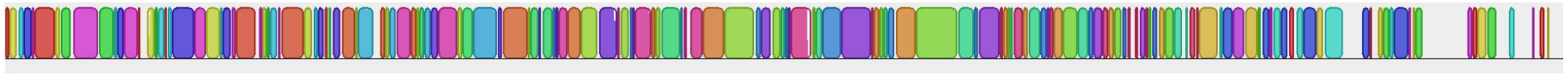
Single cryptic BGCs can be found

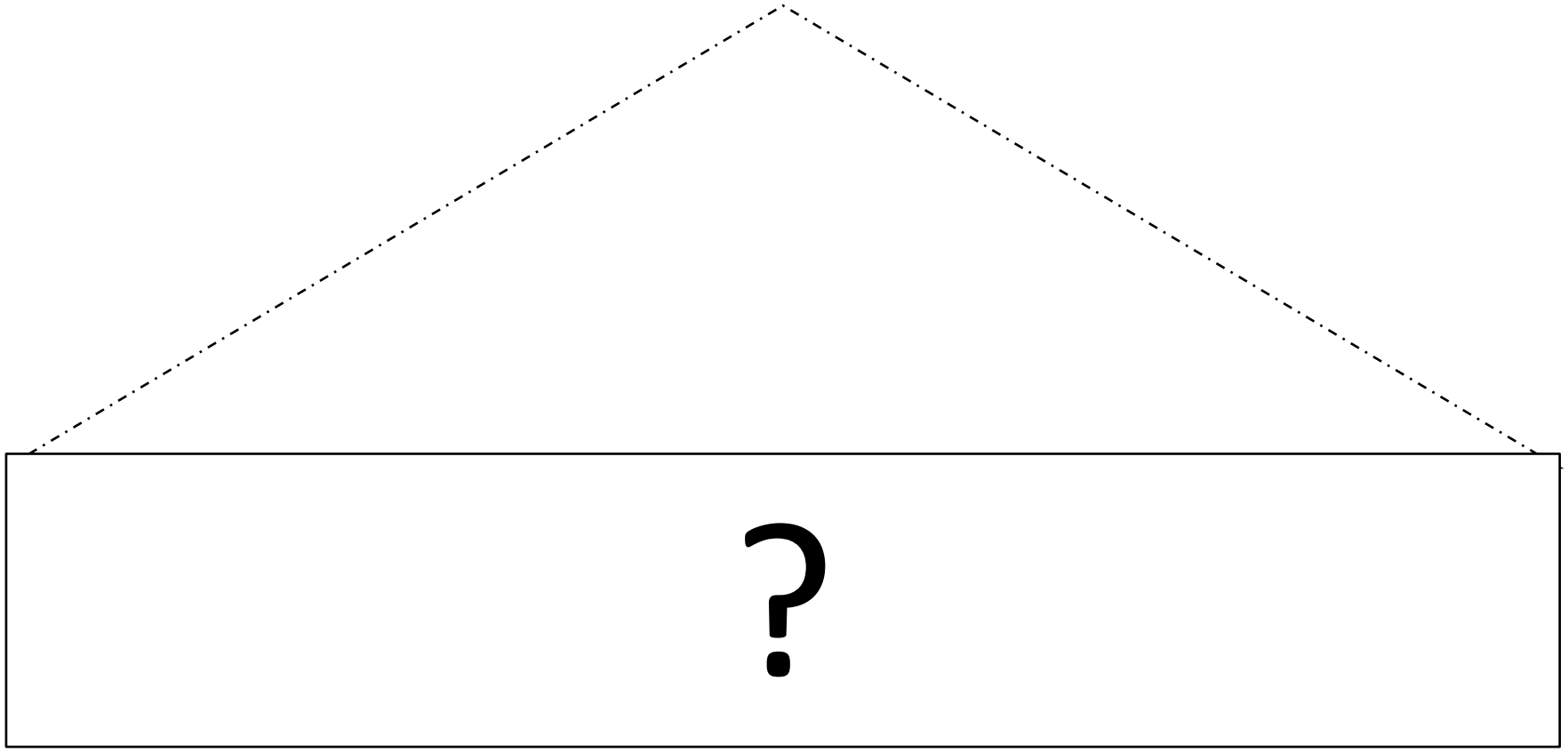
Mixta calida



No BGCs can be found

Aeromonas hydrophila





Other Current work

NCTC has provided some strains for us to use as indicators;

E. coli NCTC86 deposited by Theodor Escherich
M. luteus deposited by Alexander Fleming
E. coli colistin resistant
Pseudomonas aeruginosa
Candida auris

Screening started with these in 2018 as a variety of MSc projects. Already identified *C. auris* inhibitors from the *C. albicans* inhibitors.

Environmental library; anti-biofilm properties, immunoglobulin modification, hormone inhibitors, elicitor molecules for BGC activation, others covered by confidentiality agreements.

Cell free assays; structure determination, med-chem and development at the LSTM CDD

NIHR BRC / UCL £20,000 Research Prize (2015) antibiofilm screening;

NPRONET / BBSRC; Proof of concept study £50,000 (2018) antibiotic elicitation

Other Current work

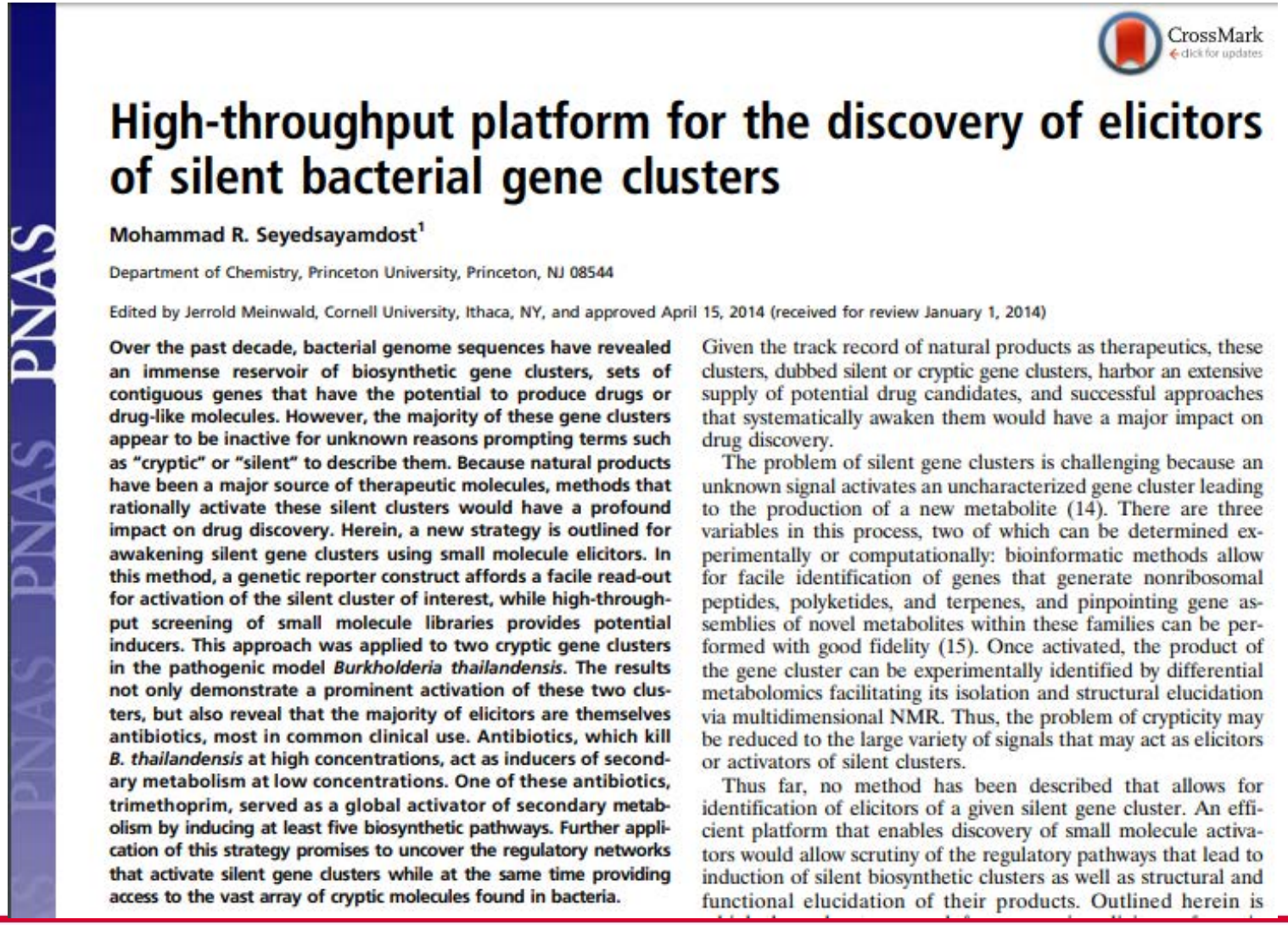
Can we encourage environmental isolates to turn on silent biosynthetic gene clusters?

Can we use small quantities of antimicrobials to turn on silent biosynthetic gene clusters?

Rather than use many elicitors on a single bacterial strain I wanted to use many bacterial strains against a small number of elicitors.

Tetracycline, Ciprofloxacin, Ampicillin, Clindamycin, Triclosan, CTAB.

Different mechanisms and targets, natural and synthetic.



PNAS PNAS PNAS

CrossMark click for updates

High-throughput platform for the discovery of elicitors of silent bacterial gene clusters

Mohammad R. Seyedsayamdost[1]

Department of Chemistry, Princeton University, Princeton, NJ 08544

Edited by Jerrold Meinwald, Cornell University, Ithaca, NY, and approved April 15, 2014 (received for review January 1, 2014)

Over the past decade, bacterial genome sequences have revealed an immense reservoir of biosynthetic gene clusters, sets of contiguous genes that have the potential to produce drugs or drug-like molecules. However, the majority of these gene clusters appear to be inactive for unknown reasons prompting terms such as "cryptic" or "silent" to describe them. Because natural products have been a major source of therapeutic molecules, methods that rationally activate these silent clusters would have a profound impact on drug discovery. Herein, a new strategy is outlined for awakening silent gene clusters using small molecule elicitors. In this method, a genetic reporter construct affords a facile read-out for activation of the silent cluster of interest, while high-throughput screening of small molecule libraries provides potential inducers. This approach was applied to two cryptic gene clusters in the pathogenic model *Burkholderia thailandensis*. The results not only demonstrate a prominent activation of these two clusters, but also reveal that the majority of elicitors are themselves antibiotics, most in common clinical use. Antibiotics, which kill *B. thailandensis* at high concentrations, act as inducers of secondary metabolism at low concentrations. One of these antibiotics, trimethoprim, served as a global activator of secondary metabolism by inducing at least five biosynthetic pathways. Further application of this strategy promises to uncover the regulatory networks that activate silent gene clusters while at the same time providing access to the vast array of cryptic molecules found in bacteria.

Given the track record of natural products as therapeutics, these clusters, dubbed silent or cryptic gene clusters, harbor an extensive supply of potential drug candidates, and successful approaches that systematically awaken them would have a major impact on drug discovery.

The problem of silent gene clusters is challenging because an unknown signal activates an uncharacterized gene cluster leading to the production of a new metabolite (14). There are three variables in this process, two of which can be determined experimentally or computationally: bioinformatic methods allow for facile identification of genes that generate nonribosomal peptides, polyketides, and terpenes, and pinpointing gene assemblies of novel metabolites within these families can be performed with good fidelity (15). Once activated, the product of the gene cluster can be experimentally identified by differential metabolomics facilitating its isolation and structural elucidation via multidimensional NMR. Thus, the problem of crypticity may be reduced to the large variety of signals that may act as elicitors or activators of silent clusters.

Thus far, no method has been described that allows for identification of elicitors of a given silent gene cluster. An efficient platform that enables discovery of small molecule activators would allow scrutiny of the regulatory pathways that lead to induction of silent biosynthetic clusters as well as structural and functional elucidation of their products. Outlined herein is

Elicitation conclusions

Elicitation using small quantities of antibiotics may be a useful approach for large screening programmes BUT it must be done in conjunction with a “without elicitor” screen to avoid loss of BGC products.

Need to discount synergy between antibiotic elicitors and NPs; gives us an the possibility of finding antibiotic potentiators if we are not observing elicitation.

Molecular studies are ongoing

A. nature of the molecules being produced
B. mechanisms off elicitation / potentiation

Final Conclusions

Swab and Send has become a self-sustaining (in terms of interest and finance) long term (> 3 years) crowd-funded, citizen science AMR and DD project which is able to reach a wide and varied audience.

Resulted in bacterial isolates of genuine scientific interest along multiple lines of investigation

Resulted in one of the most random environmental isolate collection in the UK (Europe?)

Resulted in numerous collaborations and further funding opportunities (too much for one career)

Acknowledgements

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Mr Diderik van Halsema
Ms Karen Brady

Dr Liam Reynolds UCL
Ms Kimie Rosendahl UCL
Prof Nigel Brown
Dr Jenny Rohn UCL
Dr Adam Rutherford
Dr Chris van Tulleken
Mrs Maryn McKenna
Mr David Abramovich
Dr Julie Russell

Sunday, 31 March Mother's Day 2019 in United Kingdom…