Panel 4

Marc Ouellette

Jo-Anne Dillon

Gerry Wright

Robert Hancock

Thomas Louie

What can we do to create more innovative solutions to counteract loss in antimicrobial effectiveness through research and development, new diagnostics and finding new antibiotics?

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

Discoveries for life / Découvertes pour la vie

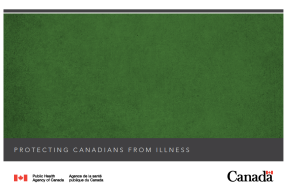
Innovation in AMR Research and Development in Canada

Marc Ouellette, FCAHS
Scientific Director Institute of Infection and Immunity
Ottawa, Sep 2017

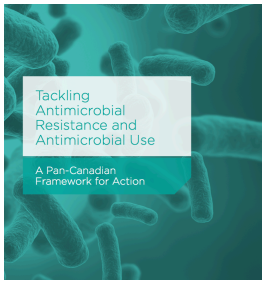
3

Surveillance-Stewardship-Innovation

2014



2015



2017

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4

Surveillance-Stewardship-Innovation

Figure 2: **One Health Linkages of Antimicrobial Resistance**

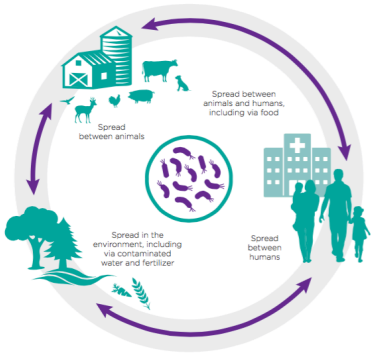
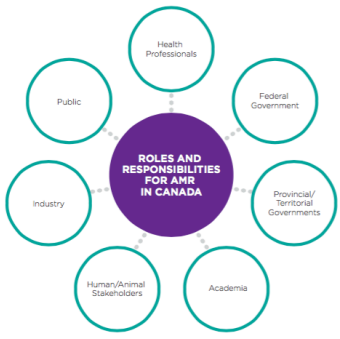


Figure 3: **Roles and Responsibilities**



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Research & Innovation Task Group (RITG)

5

MANDATE

To provide advice and recommendations on priority research and innovation activities that will increase scientific knowledge to advance the understanding AMR in humans, animals, agriculture and agri-food, in order to identify faster and effective diagnostics, and new alternative treatments that will preserve the effectiveness of antimicrobials in treating infectious diseases.

Dr. Marc Ouellette (co-chair)	Scientific Director, Institute of Infection and Immunity, Canadian Institutes of Health Research
Dr. Cheryl Waldner (co-chair)	Professor, Epidemiology Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan
Dr. Patrice Allibert	President and CEO, GenePoC
Dr. Reynold Bergen	Science Director, Beef Cattle Research Council
Dr. Eric Brown	Professor, Department of Biochemistry and Biomedical Science, McMaster University
Dr. Robert Hancock	UBC Killam Professor and Canada Research Chair, Department of Microbiology and Immunology, University of British Columbia
Dr. Philippe Lagace- Wiens	Assistant Professor of Medical Microbiology and Infectious Diseases, University of Manitoba
Dr. Joseph Rubin	Assistant Professor, Veterinary Microbiology Western College of Veterinary Medicine University of Saskatchewan
Dr. Sameeh Salama	Fedora Pharmaceuticals Inc.
Dr. Lakshmi Krishnan	Director, R&D and Program Leader. National Research Council Canada
Jennifer Van Gerwen	Animal Health Coordinator, Office of the Chief Veterinary Officer, Ontario Ministry of Agricultural, Food and Rural Affairs

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Research and Innovation Opportunities for Action:

- Support a cross-sectoral, multidisciplinary research network to facilitate antimicrobial discovery, best practices, behavioural research and economic and production impacts across sectors and jurisdictions.
- Explore mechanisms to develop the capacity and appropriate infrastructure required to further support the development of human and veterinary medicines and alternative tools.
- Establish a fast-tracked cost-effective process for licensing antimicrobial drugs, alternatives to antimicrobials and new diagnostic tools in Canada to incentivize pharmaceutical investment without compromising safety, efficacy and quality.

COMPONENT 4: RESEARCH AND INNOVATION

Responses to AMR must be evidence-based and will require increased knowledge, innovative tools and collaborative approaches to better understand resistance and the development of new treatments and strategies.

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III Strategic Plan 2013-2018

Two broad priority research areas

Priority Research Area 1: ***Preparing for and responding to emerging threats***
- **antimicrobial resistance**;
- existing and emerging microbial threats;
- environmental threats;
- vaccine development;
- improved diagnostics.

Priority Research Area 2: ***Integrating Infection and Immunity Knowledge in the Control and Prevention of Chronic Disease.***
- Inflammation;
- human microbiome;
- transplantation;
- human immunology and immunotherapy;
- environmental threats;
- Chronic viral infections including HIV/AIDS and hepatitis C.

8

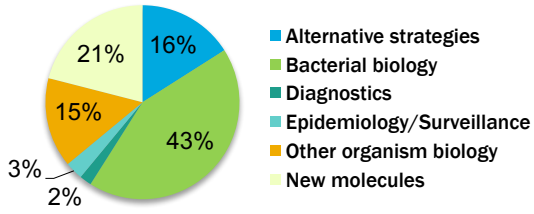
CIHR Response to AMR

AMR Investments - 2000 to 2016
$249M

Investigator-initiated
$185M

Priority-driven
$64M

5 Year Investment
2010-11 to 2014-15



9

CIHR Strategic Investments

Investments (millions $)	2011-12	2012-13	2013-14	2014-15	2015-16	Total
Antimicrobial resistance	19,9 $	19,8 $	17,9 $	18,8 $	20,2 $	**96,6 $**
Existing and emerging threats	21,9 $	32,2 $	36,5 $	48,7 $	62,8 $	**202,0 $**
Vaccines	19,2 $	13,3 $	13,9 $	18,0 $	17,6 $	**82,1 $**
Inflammation	36,4 $	49,5 $	59,8 $	68,3 $	59,0 $	**273,1 $**
Human Microbiome	3,8 $	5,2 $	7,5 $	9,2 $	15,3 $	**41,0 $**
Transplantation	15,0 $	17,1 $	20,5 $	20,3 $	20,7 $	**93,6 $**
Human immunology and immunotherapy	40,6 $	52,1 $	58,1 $	59,9 $	56,4 $	**267,2 $**
HIV/AIDS	40,6 $	52,1 $	58,1 $	59,9 $	56,4 $	**267,2 $**
Hepatitis C	8,6 $	8,5 $	8,6 $	12,6 $	12,1 $	**50,5 $**

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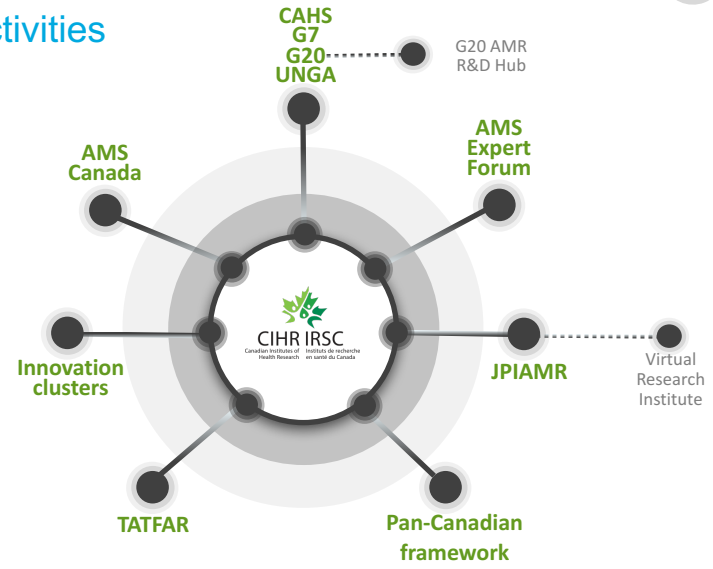
AMR Activities



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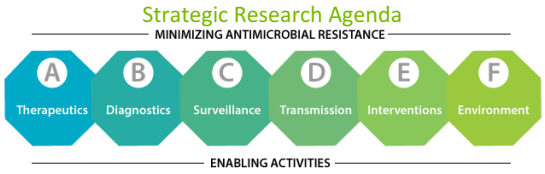
CIHR Actions – International Partnerships

JPIAMR joins forces across nations to fight AMR through effective collaborative actions in areas of unmet needs.



Canada, through CIHR is a participating country in the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), a consortium of 26 member countries

12

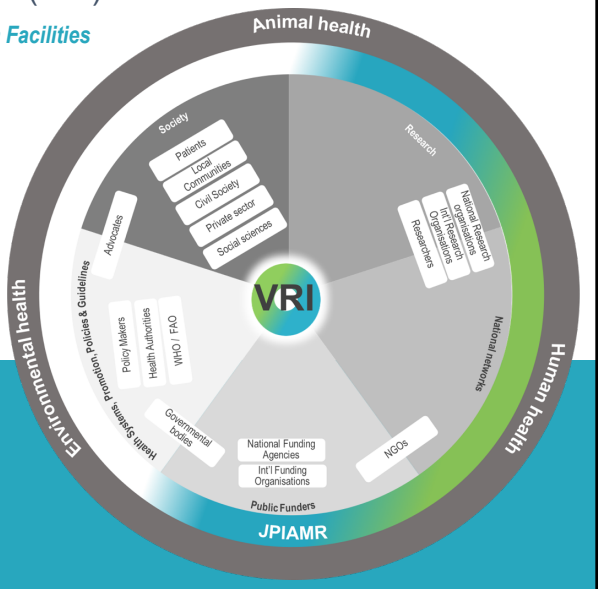
CIHR recent Investments in AMR

Funding opportunity	Launch Date	CIHR Investment	Funding start date	Topics
JPIAMR InnovaResistance 12 countries	January 2014	$3.8M for 3 years	January 2015	Innovative approaches to AMR
JPIAMR Transmission Dynamics 18 countries	January 2016	$2.6M for 3 years	January 2017	Transmission One Health Intervention
JPIAMR Working Groups on AMR 9 countries	February 2016	$110K for 1 year	January 2017	White papers on priority topics
AMR Point of Care Diagnostics in Human Health	May 2016	$1.39M for 2 years	January 2017	Diagnostics
JPIAMR Prevention and Intervention 15 countries	January 2017	$3M for 3 years	October 2017	Stewardship One Health

13

Virtual Research Institute (VRI)

A Dynamic Network of AMR Research Facilities



jpiamr
Joint Programming Initiative on Antimicrobial Resistance

One-Health Approach

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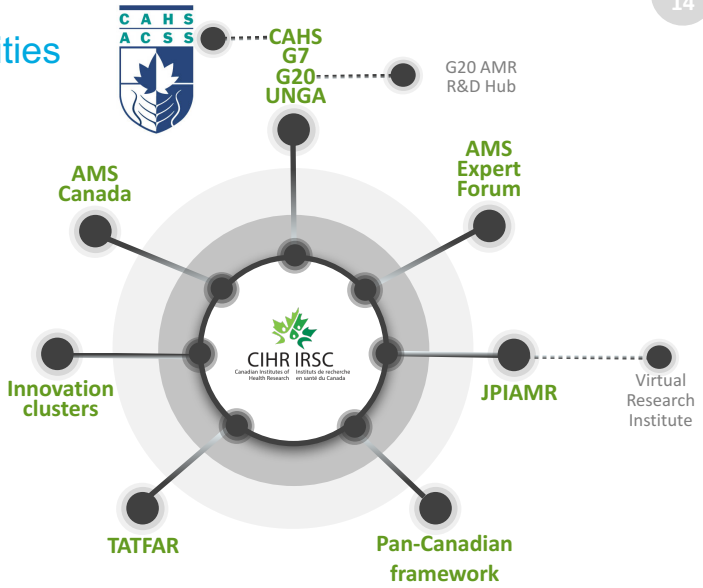
AMR Activities



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Canadian Academy of Health Sciences
Académie canadienne des sciences de la santé

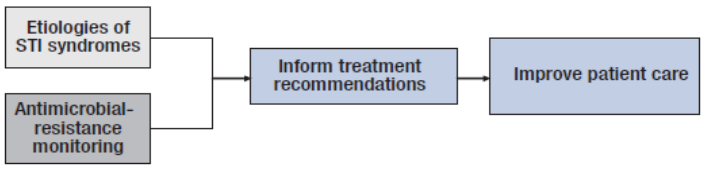
What can we do to create more innovative solutions?

PANEL 4

NEW DIAGNOSTICS AND DIAGNOSTIC APPROACHES

Jo-Anne R Dillon PhD FCAFS FRSC
Distinguished Professor and Head, Dep't Microbiology & Immunology, College of Medicine, University of Saskatchewan
Research Scientist, Vaccine & Infectious Disease Organization –International Vaccine Center, University of Saskatchewan

Antimicrobial Susceptibility Testing (AST)



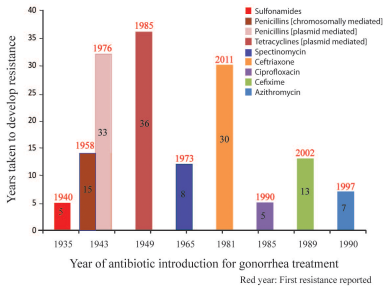
Effectively use current antibiotics and optimize treatment

Detect emerging resistance

Ascertain mechanisms of resistance to inform the development of new antimicrobial agents

Accurate AST needed for effective antimicrobial stewardship

Evaluate the clonal spread of AMR
- Presently difficult to achieve in low and middle income countries



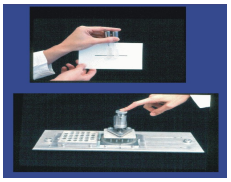
Dillon et al Culture 2015 35:1

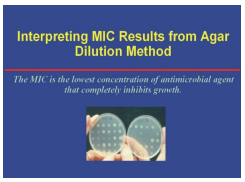
Detecting Antimicrobial Resistance (AMR): a Global Health Priority

AST Methods:

Broth Dilution, Disc Susceptibility, Agar Dilution, Etest

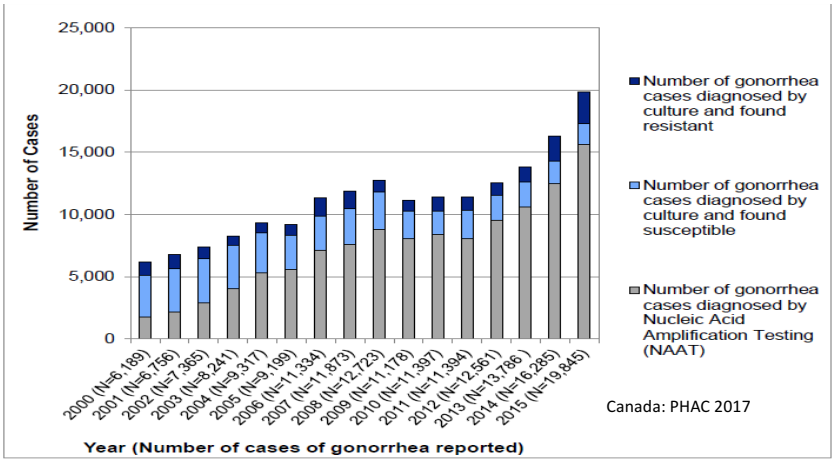
	Agar Dilution	Etest
Methodology	Difficult, labour intensive	Easier, requires training
	Technology >40 years old	
Type of Laboratory	Used primarily in reference laboratories	Reference mostly
Cost	Technical costs	Unaffordable in many settings. May not be available.
QA/QC programs	Required but few engage	Required but few engage





Challenges for AMR Diagnosis (of Ng) in High-income Countries:

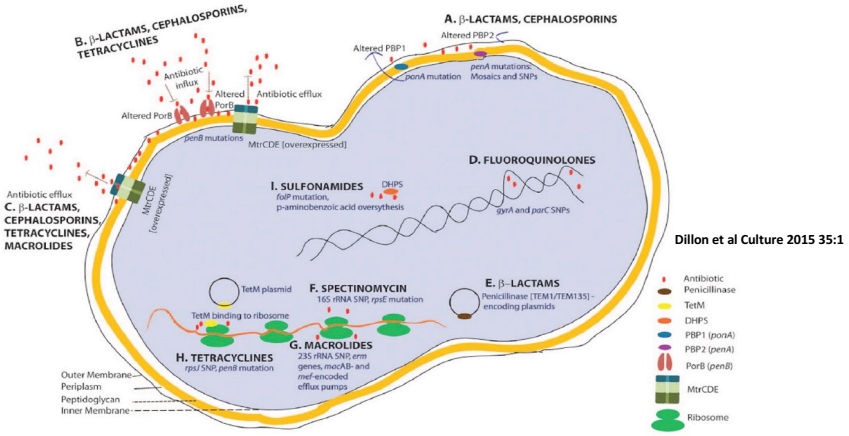
Identification of Pathogens (e.g. STIs) by Nucleic Acid Amplification Testing (NAAT)
no AMR testing done. Molecular testing needed



Canada: PHAC 2017

Molecular Antibiograms: PCR and WGS-based Methods

Detecting Clinically Relevant Antimicrobial Resistance



Dillon et al Culture 2015 35:1

Resistance Mechanisms in *N. gonorrhoeae*

PCR-Based Diagnostics of AMR

Performance of Molecular (PCR-DNA sequencing) Assays for AMR:

Can standardization of tests for low and middle Income countries be achieved?

SNP	Ceftriaxone MIC ≥0.032 mg/L		Cefixime MIC ≥0.032 mg/L	
	% Sensitivity	% Specificity	% Sensitivity	% Specificity
ponA	98.0%	72.8%	95.8%	66.7%
mtrR	94.6%	80.4%	92.4%	73.8 %
porB	93.3%	64.8%	90.9%	57.7%
penA Mosaic	61.1%	99.0%	63.2%	99.1%

SNP	Ciprofloxacin MIC ≥1 mg/L	
	% Sensitivity	% Specificity
gyrA S91	100%	93.8%
gyrA D95	100%	96.9%
parC	96.1%	99.0%

Sensitivity and Specificity of SNP targets on 252 *N. gonorrhoeae* cultures from Canada

Peterson et al JCM 2015 53:2042; Peterson et al JCM 2015 53:3606

Whole Genome Sequencing (WGS)

Limited number of WGS studies to analyze gonococcal AMR

Advantages: all genetic determinants, including epidemiological typing genes and identification sequences can be ascertained simultaneously

Still relatively expensive, labour intensive and needs expertise; requires specialized analysis

Analyses (phylogenetic trees etc.) are generally not focussed on clinical outcomes at present

Costs are declining and diagnostic-friendly platforms are being developed

Research required to ascertain effects on susceptibility of emerging AMR determinants

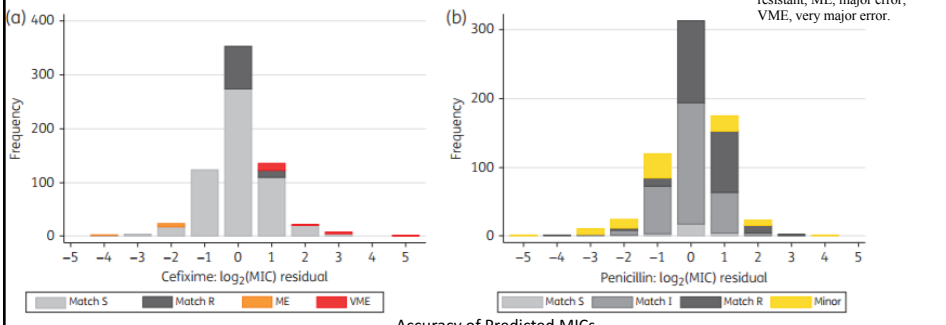
J Antimicrob Chemother 2017; **72**: 1937–1947
doi:10.1093/jac/dkx067 Advance Access publication 10 March 2017

Journal of Antimicrobial Chemotherapy

WGS to predict antibiotic MICs for *Neisseria gonorrhoeae*

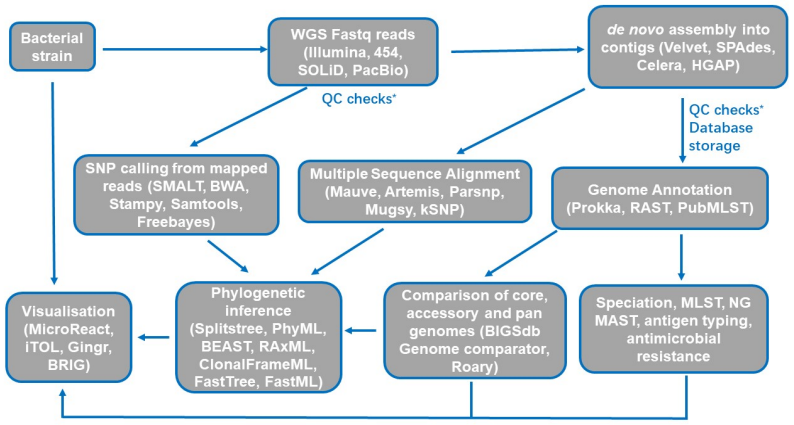
David W. Eyre[1–3]*, Dilrini De Silva[1–3], Kevin Cole[4,5], Joanna Peters[4,5], Michelle J. Cole[6], Yonatan H. Grad[7,8], Walter Demczuk[9], Irene Martin[9], Michael R. Mulvey[9], Derrick W. Crook[1–3,5], A. Sarah Walker[1–3], Tim E. A. Peto[1–3] and John Paul[2,4,5]

S, susceptible;I, intermediate; R, resistant; ME, major error; VME, very major error.



Accuracy of Predicted MICs

Development of Bioinformatic Pipelines for Clinical WGS Applications



Harrison O et al. *Pathog Dis* 2017 ftx060. doi:10.1093/femspd/ftx060

Applications of Molecular Testing:

Point-of-Care (POC) Diagnostic Testing

- Most POC tests must meet ASSURED criteria, i.e. affordable, sensitive, specific, user friendly, rapid, robust, equipment-free, deliverable
  - Definition is product oriented and restrictive
- Alternative definition (Pai et al Microbe 2015, 10:103): Diagnostic testing that will result in a clear and actionable management decision such as when to start treatment
  - Goal oriented
  - POC is a spectrum of technologies, users and settings
  - Not defined by technology but the diagnostic process that leads to a rapid completion

Point-of Care (POC) Tests for AMR: Testing for Susceptibility?

POC tests being evaluated for treatment with ciprofloxacin for many organisms

POC tests for penicillin susceptibility in *N. gonorrhoeae*?

- Penicillin is used for treating Ng infections in regions of Australia
- Comprehensive molecular testing for penicillin resistance determinants
- A similar test strategy in Saskatchewan (<5% resistance) might allow the use of penicillin in regions with low resistance

Clinical Infectious Diseases 2016, 64:1268-70

BRIEF REPORT

Implementation of a Rapid Genotypic Assay to Promote Targeted Ciprofloxacin Therapy of *Neisseria gonorrhoeae* in a Large Health System

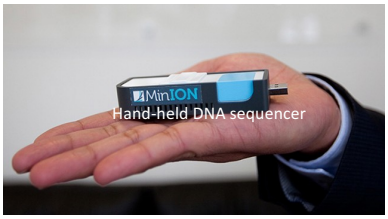
Lao-Tzu Allan-Blitz,[1] Romney M. Humphries,[2] Peera Hemarajata,[2] Ashima Bhatti,[3] Mark W. Pandori,[4] Mark J. Siedner,[5] and Jeffrey D. Klausner[6,7]

The Future: POC/ WGS-based Diagnostic ID and AST

WGS with portable DNA sequencers (e.g. MinION and others)

Can both a pathogen and its AMR be detected?

- Yes, multiplexing in NAAT and possibly other formats
- Such tests formats could be user- and "field"- friendly



Sample preparation → 105 min → DNA extraction → 30 min → Library preparation → 15 min

MinION sequencing → 60 min → Bioinformatic analysis → 30 min → Results
Bacterial & Resistance genes I/D

JAC 2017 72:104-114

Other Diagnostic Approaches for AMR under Development

Microfluidic applications

Encapsulation of bacterial cells in micro and nanodroplets with microfluidic manipulation

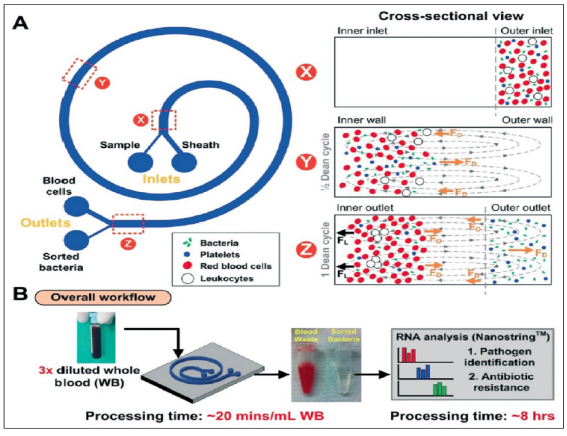
Leveraging enhanced ultrasensitive detection methods to relay phenotypic information more rapidly

- Mechanical transducers: Atomic force microscopy –mechanical readout of changes to the cell membrane
- Electrochemical sensors – analysis of rRNA and possibly resistance genes
- Electrochemical bacterial phenotyping
- Matrix-assisted laser desorption Time-of-Flight Spectromotry (MALDI-TOF)

Fluorescence In Situ Hybridization (FISH)

Addition of microsocopy to various methods

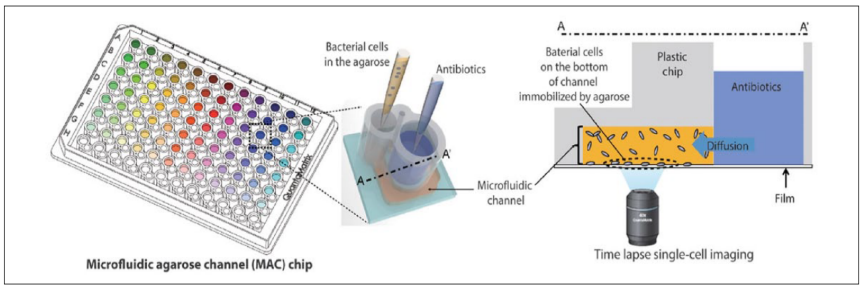
Microfluidics



SLAS Tech 2017 22:113-121

- Microfluidics: manipulating and controlling fluids, usually in the range of microliters (10^{-6}) to picoliters (10^{-12}), in networks of channels with dimensions from tens to hundreds of micrometers
- Miniaturizes biological assays
- Gradient microfluidics allows rapid bacterial growth inhibition testing

Rapid AMR Testing and Bacterial Identification: Single Cell Methods

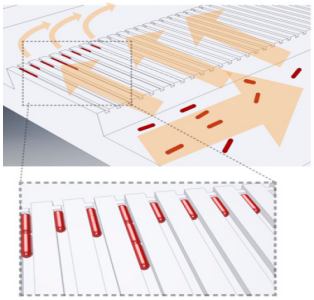


Monitoring Cell Morphology –

High Throughput Agarose Channel Chip

SLAS Tech 2017 22:113-121

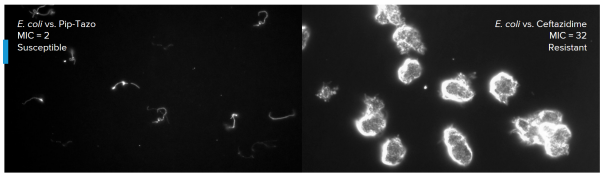
AST using Cell Imaging Methods: Different (POC) Strategies



Microfluidic chip and cell growth monitored by single cell imaging microscopy –AST in 30 minutes

PNAS 2017, 114:9170-9175

Morphokinetic Cellular Analysis



Accelerate Diagnostics Inc

Final Thoughts

Many innovations for the rapid and accurate diagnosis of AST

Commercialized strategies with other innovations being actively developed

Standardized AST formats would not require culture and would simultaneously identify pathogens

Molecular/WGS AMR testing in low and middle income countries may be simpler than MIC testing

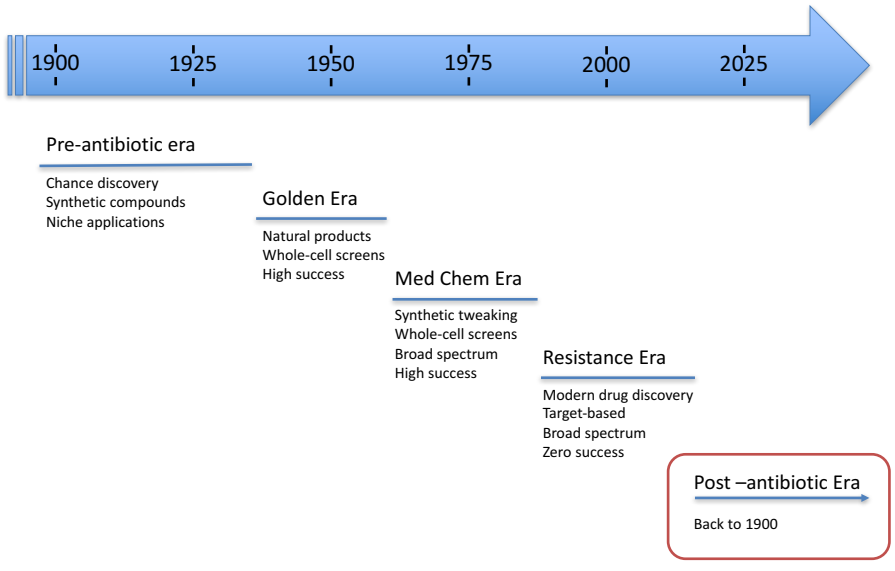
- Costs of WGS decreasing and becoming competitive
- Bioinformatic platforms can be distributed internationally
- Considerable international standardization and collaboration required

Thank you

Innovation in New Antibiotics

Gerry Wright

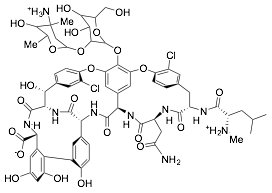
Michael G. DeGroote
INSTITUTE FOR INFECTIOUS DISEASE RESEARCH



Brown & Wright (2016) *Nature* **529**: 336

Why do we need new antibiotics?

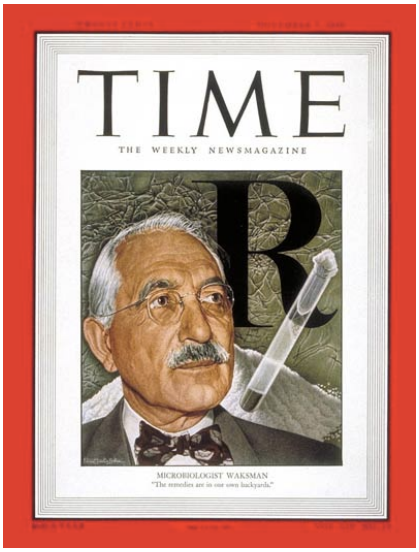
- No antibiotics have proven to be resistance proof
- Resistance is ancient
- Resistance is transferred vertically (mother to daughter) and horizontally (cell to cell)
- We cannot overcome evolution



Nature (2011)**477**:457-61

'Old' Antibiotics

- Penicillin & streptomycin ushered in the 'Golden Era' of antibiotic discovery
- Natural products isolated from other microbes proved highly effective and generally non-toxic
- By the mid 1960s, new antibiotics proved difficult to find
- Next two decades dominated by medicinal chemistry to improve efficacy and overcome resistance

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JANUARY 1, 1949
Effective local Penicillin Therapy maintained for one day WITH 3 PIECES
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PENICILLIN CHEWING GUM A&H
In Boxes of Six Pieces 3/-
Each piece contains 5,000 International Units of Penicillin (Calcium Salt)
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Index to Advertisers pp. 3 & 6
Clinically Proven Deodorant
UNBREAKABLE PLASTIC BOTTLE
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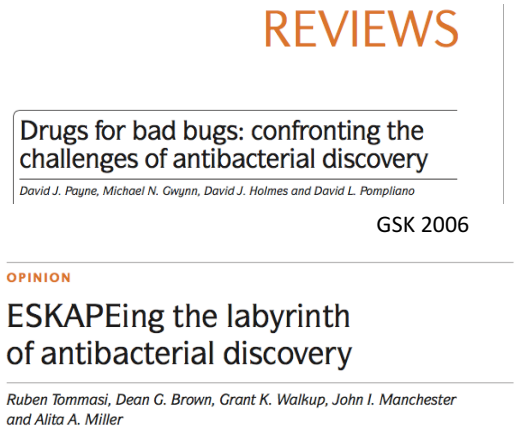
ROLL-ON DEODORANT
Revlon
ANTI-PERSPIRANT
'Hi and Dri'
FORTIFIED WITH NEOMYCIN
ROLL-ON DEODORANT
SORE THROAT?
Antibiotic Candettes give immediate soothing relief!
CANDETTES work 2 ways:
1 Double Antibiotic action...fights germs! Not just one—but two safe, proven antibiotics kill many irritation-causing throat germs, on contact!
2 Anesthetic action...relieves soreness! A safe and effective anesthetic acts instantly to relieve soreness of inflamed membranes.
Not an ordinary cough drop—delicious, orange-flavored Candettes are a proven medication! Get them at your drug store.
Guaranteed by Good Housekeeping
Candettes
Candettes
By the World's Largest Producer of Antibiotics


Increase Calf Growth Rate!
AUREOMYCIN
Rapid Development, Little or No Scours, Improved Health
Reported When Rations Are Fed that Contain This Golden Antibiotic
An astounding step-up in feed utilization by calves, based on use of AUREOMYCIN in manufactured feeds, is now under way. Present evidence points to spectacular increases in rate of growth, marked reductions in calf losses from intestinal troubles and pronounced improvement in general health and appearance, when rations include aureomycin.
Nutritional studies that included field tests under farm conditions have been made by Agricultural Experiment Stations. These involved both pure aureomycin and Vitamin B12 and Antibiotic (Aureomycin) Feed Supplement. It was found that diets that contained aureomycin produced increases of 10, 20 and 30 per cent (in certain instances, considerably higher percentages) in rate of calf growth. Little or no scouring was encountered and improvement in general well-being of tested calves over control groups was marked.
Several studies that were continued well into the period of active rumination revealed no interference with rumen function nor any harmful effects, even when levels of aureomycin were fed at levels many times higher than in commercially practical.
Availability of aureomycin in manufactured calf starters, feeds and supplements offers dairy farmers, stockmen and breeders not only an opportunity to mature their calves faster and more economically, but also to cut down or escape the crippling calf losses from intestinal troubles that have been sapping the nation's calf crops for many years.
Aureomycin has been proved highly effective for swine, chickens, turkeys, calves and several kinds of small animals and is today the antibiotic most widely used in the feed field. Ask for a feed that contains aureomycin!
Animal Feed Department
LEDERLE LABORATORIES DIVISION
30 Rockefeller Plaza
New York 20, N. Y.

Genomics and target-based drug discovery

- Advent of genome sequencing, structure-based drug design, and combinatorial chemistry promised a new route to antibiotics
- No new drugs



REVIEWS

Drugs for bad bugs: confronting the challenges of antibacterial discovery

David J. Payne, Michael N. Gwynn, David J. Holmes and David L. Pompliano

GSK 2006

OPINION

ESKAPEing the labyrinth of antibacterial discovery

Ruben Tommasi, Dean G. Brown, Grant K. Walkup, John I. Manchester and Alita A. Miller

AstraZeneca 2015

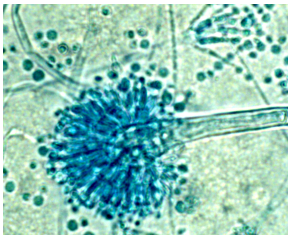
Now what?

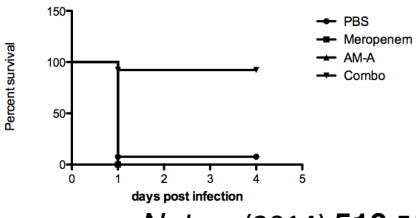
Back to the future: Natural Products

- New antibiotics – new sources
- Discarded known antibiotics (narrow spectrum)
- 'Cryptic' compounds
- Synthetic biology for novel compounds

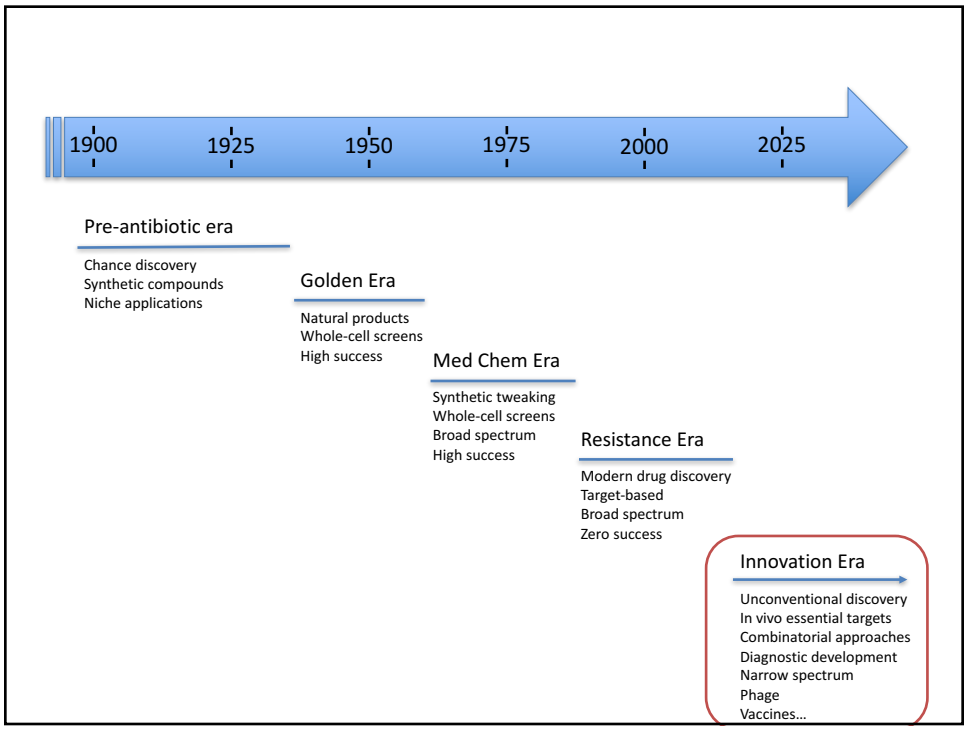
Rescuing 'old' antibiotics: Antibiotic Adjuvants

- Adjuvants are not antibiotics
- Block resistance
- Enhance antibiotic activity



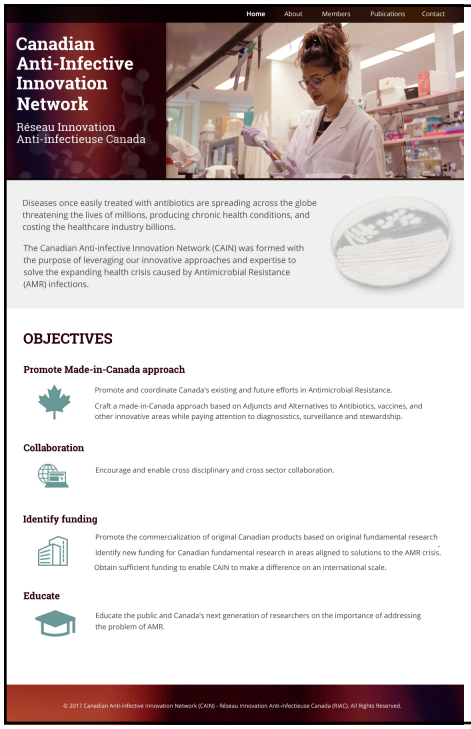


Nature (2014) **510**:503-06



Innovation Era

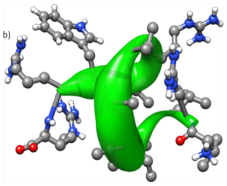
- Unconventional discovery
- Antibiotic adjuvants
- In vivo essential targets
- Combinatorial approaches
- Diagnostic development
- Narrow spectrum compounds
- Phage
- Vaccines…



Partnership between:

- Academia
- Private Sector
- Not-for Profit Sector
- Government

www.cain-amr.ca



Canadian Academy of Health Sciences
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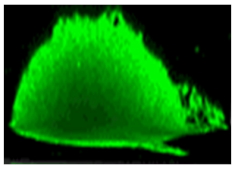
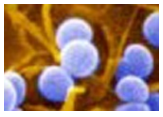
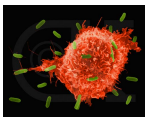


What Innovations R&D & Diagnostics?





R.E. W. (Bob) Hancock

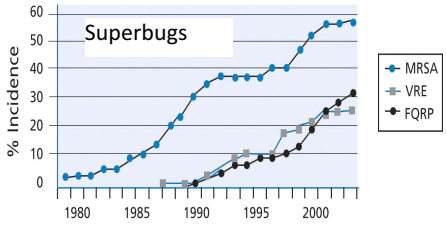
Department of Microbiology and Immunology
University of British Columbia

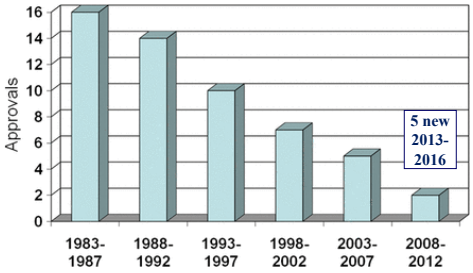
Panel 4

Conflicts of Interest: Bob Hancock is the founder of 2 virtual companies ABT Innovations and Sepset Biotherapeutics that are developing new peptides as alternatives to antibiotics and sepsis diagnostics respectively. He consults with many companies re antimicrobials.

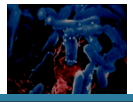
The Antibiotic Development Paradigm is Fractured

- Antibiotics are the single most successful medical intervention
- No antibiotics would lead to difficulties with major surgeries, cytotoxic therapy, transplantations, early-term births & even minor injuries.
- ↑↑↑Antibiotic resistance
- Dry pipeline; Traditional and genomics-based antibiotic development has not worked well.





There is an urgent need for alternative strategies to treat infections



When do antibiotics fail?

1. Acquired Resistance (mutations/plasmids etc)
2. Sepsis (~30% death rate; 5-17 million deaths/yr worldwide).

3. Chronic Infections especially biofilms (65% of all infections and very resistant).
4. Individuals with disturbed immune systems (chemotherapy, immunosuppressive disease, genetic diseases, massive injuries or burns).

The last 3 indications are rarely considered when developing new anti-infectives

Alternatives to antibiotics (Adjuncts/Adjuvants)

Alternatives to antibiotics—a pipeline portfolio review

Lloyd Czaplewski, Richard Bax, Martha Clokie, Mike Dawson, Heather Fairhead, Vincent A Fischetti, Simon Foster, Brendan F Gilmore, Robert E W Hancock, David Harper, Ian R Henderson, Kai Hilpert, Brian V Jones, Aras Kadioglu, David Knowles, Sigríður Ólafsdóttir, David Payne, Steve Projan, Sunil Shaunak, Jared Silverman, Christopher M Thomas, Trevor J Trust, Peter Warn, John H Rex

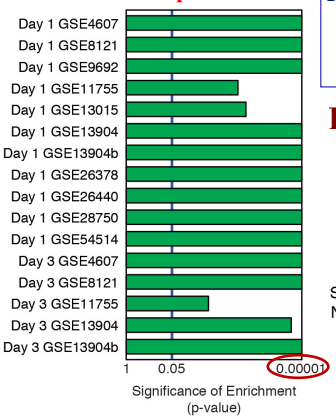
Antibiotics have saved countless lives and enabled the development of modern medicine over the past 70 years. However, it is clear that the success of antibiotics might only have been temporary and we now expect a long-term and perhaps never-ending challenge to find new therapies to combat antibiotic-resistant bacteria. A broader approach to address bacterial infection is needed. In this Review, we discuss alternatives to antibiotics, which we defined as non-compound approaches (products other than classic antibacterial agents) that target bacteria or any

Lancet Infect Dis 2016; 16: 239–51
Published Online January 12, 2016
http://dx.doi.org/10.1016/S1473-3099(15)00466-1

Antibodies; Vaccines; Probiotics; Lysins; Wild Type and Engineered Bacteriophages; Immune Stimulation; Antimicrobial peptides; Innate Defence Regulators; Anti-biofilm Peptides

Diagnostics & Host directed therapies (Sepsis)

Meta-analysis of >600 patients

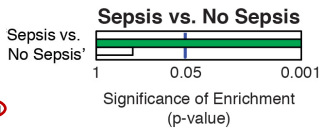


Accuracy = 97%

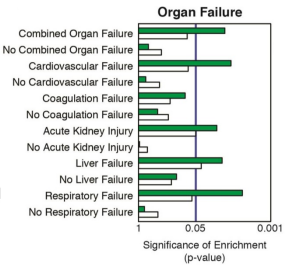
Early Sepsis is associated with a diagnostic immune non-responsiveness gene expression signature that demonstrates immune cell reprogramming (cellular amnesia)

The Signature predicts severe sepsis in the emergency ward

Clinical Study



& organ failure



Pena OM, DG Hancock et al. 2014. eBiomedicine 1:64–71.; Confirmed by Shalova, IN et al. 2015. Immunity 42:1–15.

Can we make antibiotics work better in sepsis patients by restoring normal immunity i.e. reversing cell amnesia?

Vaccines

The most cost effective Intervention

ReVAMP

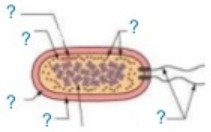
GenomePrairie

Genome BritishColumbia

Reverse Vaccinology

Bioinformatics

Identify Components



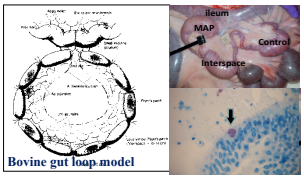
Potential Antigens

Gene Synthesis

GE³LS

GenomeCanada

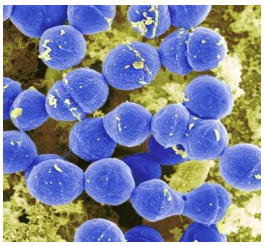
Vaccine Testing, Development & Commercialization



Testing in Cattle Animal Models

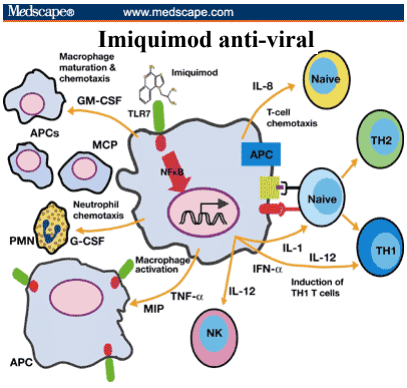
MAP gene designation	Function of product
MAP0044 (pmi-1?)	Transglycosylase
MAP0109 (mcr?)	ABC-type transport, resistance to organic solvents
MAP0110	Hypothetical protein
MAP0144	Hypothetical protein
MAP0145	Hypothetical protein
MAP0157 (PE_2)	Hypothetical protein
MAP0214	Hypothetical protein
MAP0272	Hypothetical protein
MAP0273	Hypothetical protein
MAP0326	Hypothetical protein
MAP0395c	Hypothetical protein
MAP0401	Hypothetical protein
MAP0412 (dppD?)	ABC transport system
MAP0574 (pstB)	ABC phosphate transporter
MAP0592 (phoR)	Histidine kinase
MAP0750c	Hypothetical protein
MAP0762	Hypothetical protein
MAP0765	Virulence factor Mce family protein
MAP0785 (fpr)	Predicted acyl-CoA transferases/carnitine dehydratase
MAP0872 (pbuS2)	Phosphate ABC transporter
MAP0876 (sucC)	Catalyses the interconversion of succinyl-CoA and succ
MAP1164 (pgs)	Catalyses the formation of 3-phospho-D-glyceroylphos
MAP1689c (fbpB)	Esterase, homolog of Ag85 from Mycobacterium tubercu
MAP1845c	Hypothetical protein
MAP2628c (mcr)	Predicted acyl-CoA transferases/carnitine
MAP2887	Hypothetical protein
MAP3770c	Hypothetical protein
MAP3968	Hypothetical protein
MAP4059 (hpcG)	Heat shock protein
MAP4157	Hypothetical protein
MAP4318	Hypothetical protein
MAP4341 (csd?)	N-Acetylmuramoyl-L-Alanine Amidase

Immune Modulation



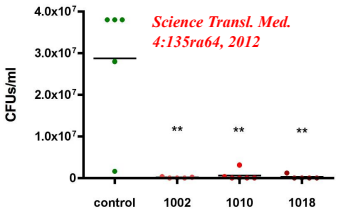
- Successful therapy against infections requires help from the host: immune response
- Immune modulation is highly used in antiviral and anticancer therapy

Hancock, R.E.W., A. Nijnik and D.J. Philpott. 2012. Modulating immunity as a therapy for bacterial infections. Nature Rev. Microbiol. 10:243-254.

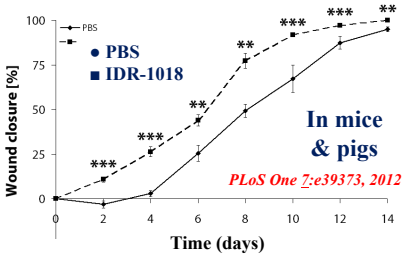


New immunomodulatory peptides show broad protection in Mouse Model Infections

Invasive *Staph. Aureus* Mouse Model



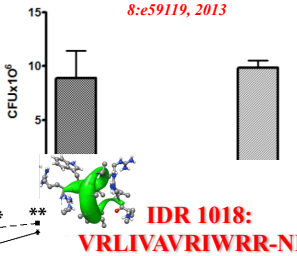
Science Transl. Med. 4:135ra64, 2012



In mice & pigs

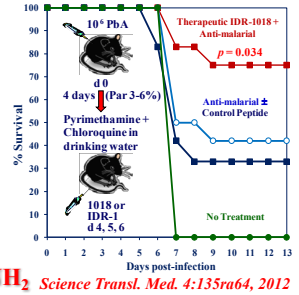
PLoS One 7:e39373, 2012

MDR -TB Mouse Model



PLoS One 8:e59119, 2013

Cerebral Malaria Mouse Model

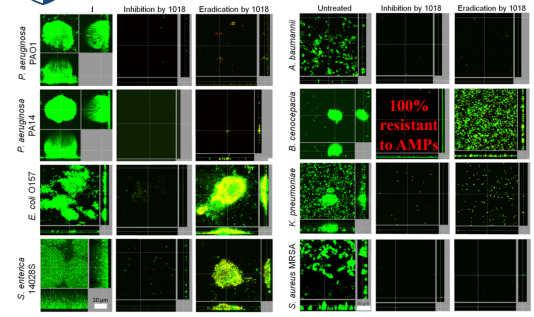


IDR 1018: VRLIVAVRIWRR-NH$_2$

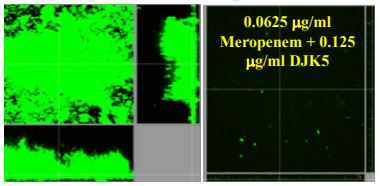
Science Transl. Med. 4:135ra64, 2012

Also protect in animal models vs. *E. coli, Pseudomonas,* MRSA, *Salmonella, Klebsiella,* Tuberculosis; Pox & HSV viruses; Sterile inflammation, Malaria, IBD; LPS/hypoxia-ischemia; → Wound Healing

Broad Spectrum Anti-Biofilm Activity when added at 0 or 2 days of biofilm formation



Carbapenemase *K. pneumoniae*

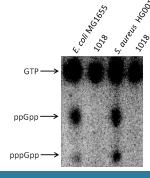


Synergy with multiple antibiotics

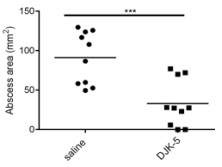
Reffuveille et al. 2014. Antimicrobial Agents Chemother. 58:5363-71.
Ribeiro et al. 2015 Antimicrob Agents Chemother, PMID: 25896694

Broad Spectrum Anti-Biofilm Activity when added at 0 or 2 days of biofilm formation

De la Fuente Nunez et al. 2014. PLoS Pathogens 10(5):e1004152



Peptides lead to rapid degradation of ppGpp (stress signal) in cells

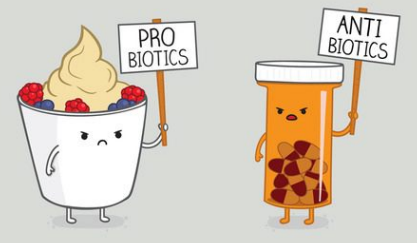


***P. aeruginosa*: 200µg intra-abscess**

Works in animal models

Sarah Mansour et al. 2016. eBiomedicine 12:219–226

Probiotics



- Use of bacteria to prevent infections
- Several mechanisms proposed, e.g. exclusion, immune modulation

Cosseau C, Hancock REW et al. 2008. Infect. Immun.76:4163-4175.

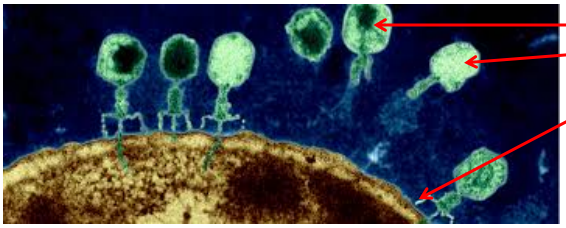
- **Already used successfully**
  - As OTC medicines: *S. salivarius* clinically proven for Strep throat, Halitosis, Chronic sore throat
  - In fecal transplantation for *C. difficile*, and
  - To reduce sepsis (24% → 15%) & enterocolitis (4.6 → 2.5%) in 10,000 premature VLBW babies (similar results in India)

BLIS Technologies

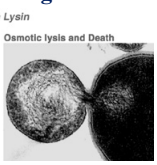
Denkel et al. 2015 Antimicrob Resistance & Infect Control 2015, 4(S1):O39

- Consortia of defined bacteria can replace normal microbiota

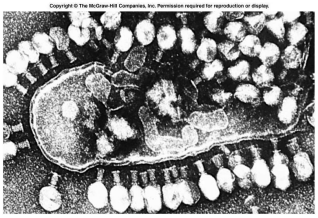
Phages and Lysins

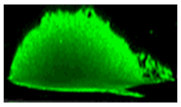


Engineered Phages
Wild Type Phages
Lysins;

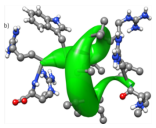


- Can use consortia of natural phages (Tblisi); phages engineered to utilize common receptors, produce Cas9-CRISPR or toxins; or lysins (enzyme from phages that destroy pathogens; Avacyn).
- Phages self propagate as long as their bacterial hosts survive.
- Several trials underway (some failures).
- More research needed to understand phage biology, create new phages with broader host ranges and detect and engineer lysins.





Conclusions



- We need new anti-infectives. Resistance is very complex. And are we doing what is required to overcome all of the situations when antibiotic therapy does not work?
- Given our dismal recent success, what if we do not discover new game-changing antibiotics?
- Alternatives to Antibiotics provide new hope.

Canadian Academy of Health Sciences
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What can we do to create more innovative solutions?

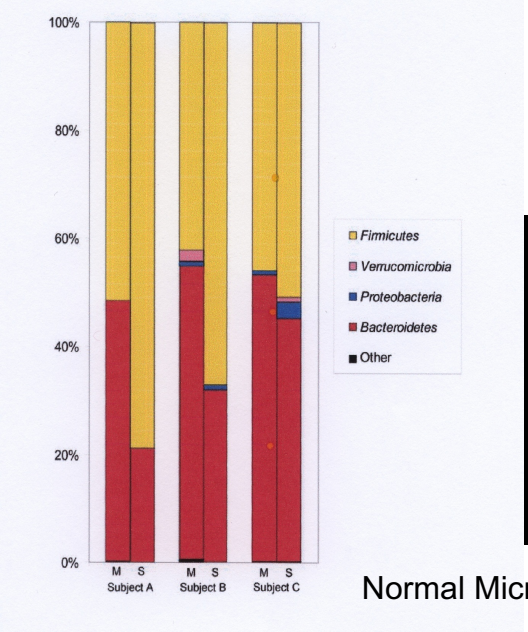
PANEL 4

Replacing Missing Microbes in heath care: Probiotics and normal flora components, some or all, for host defense.

- Not enough defense! After 7 decades of antimicrobial use (offense), with infection prevention control, antibiotic stewardship and hand hygiene (defense) to contain spread of AMR, we are loosing the war…….
- The value of *colonization resistance* has barely been utilized in health care to limit the spread of AMR. Epithelial surfaces colonized by normal microbiota resist the establishment of pathogenic microbes.
- The perfect storm! Combinations of susceptible hosts, antibiotic exposure, transmission prone health care facilities convert patients into bioreactors to amplify AMR microbes.

Why antibiotics are non-selective, deleterious to the host microbiota, creating ecologic vacuum, favoring pathogens

- ❑ intrinsic, high potency / low MICs against pathogens
- ❑ elimination pathways : liver inactivation, renal excretion, biliary excretion, intestinal secretion
- ❑ Oral antimicrobials are not necessarily safer
- ❑ While no MICs available against normal microbiota, in-vivo activity of antimicrobials shown by qPCR reveal 1000 to million fold reductions in normal microbiota



Ecklund, Bernstein, Relman. Science 2005.
Multiple colonic biopsies and feces examined by 16S rRNA sequencing

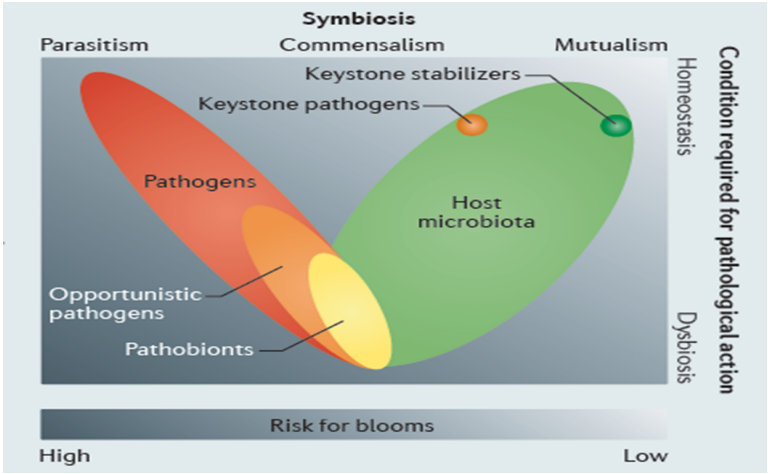
***Bacteroidetes* predominate on the mucosal surface.**

***Firmicutes* (major groupings: Clostridium coccoides /cluster XIVa and Clostridium leptum) /cluster IV [270+ genera]**

***Proteobacteria*= Gram Negative Facultatives are ~1% microbes**

Normal Microbiota holds proteobacteria in check!

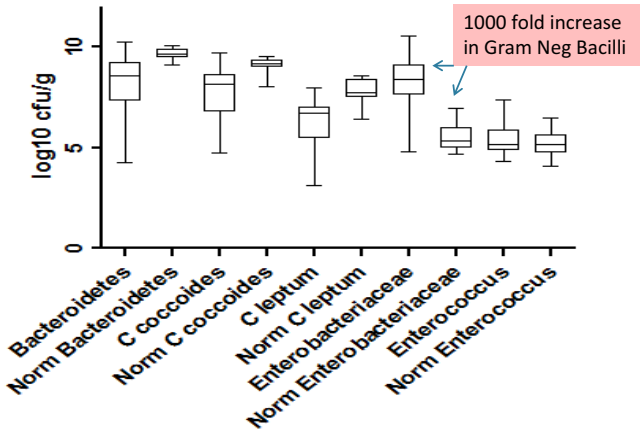
Antibiotic treatments drive the shift towards pathogen dominance



Nat Rev Microbiol 2013 Stecher et al

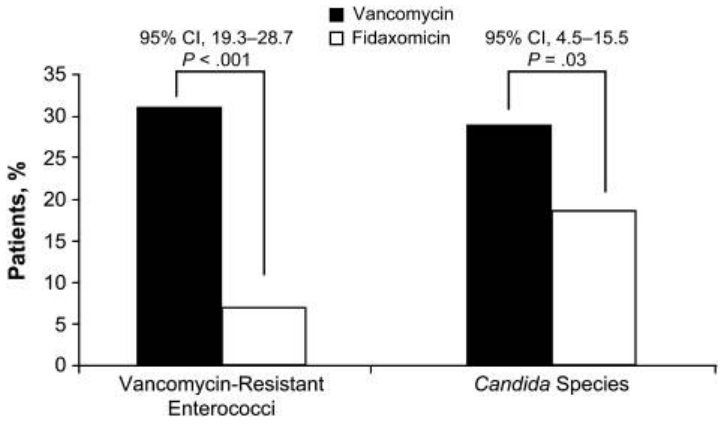
Expansion of proteobacteria in association with impairment of Bacteroidetes and Firmicutes

microbiome at CDI diagnosis vs normal controls

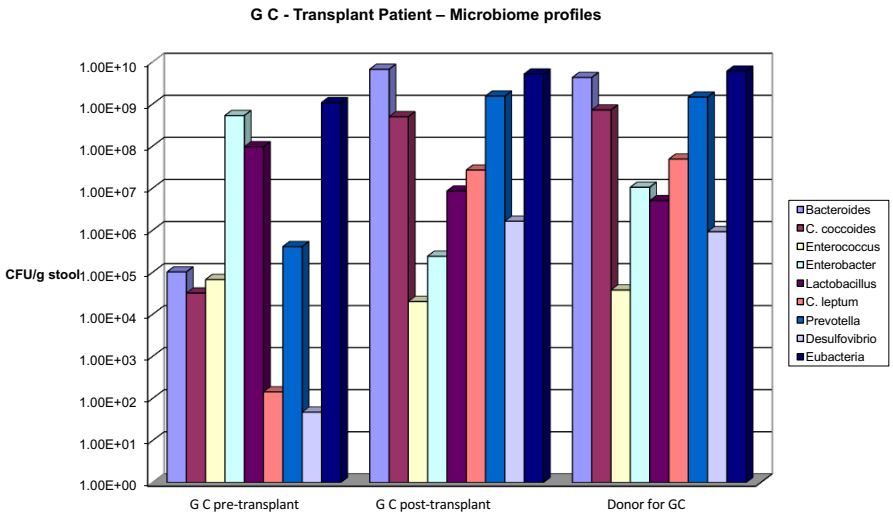


Cannon et al, J Antimicrob Chemother in press 09/2017

Healthy normal flora confers protection from acquisition of pathogens. Fidaxomicin, which preserves microflora better than vancomycin is associated with a 5 fold decrease in VRE acquisition during treatment of CDI. Nerandzic & Donskey, CID Aug 2012



FMT instantly re-complements gut microbiome in recCDI



Establishing the role of components of the microbiome for the prevention and treatment of C. difficile infection.

Rec CDI

Fecal microbiome transplantation whole stool slurry by enema, endoscope, nasojejunal, capsules

Modified FMT..pure microbial preps, microbe ID and recovery

Specific microbial 'probiotics' that restore colonization resistance in a **preventative** manner in susceptible populations

Modified FMT via subcomponents of microbiota alone or in combination

Discovery of functional role of microbial Genus / species in host defence / colonization resistance

Hot spots for Missing Microbes

reduced alpha and beta diversity of mucosal microbes

- Intensive care units
- Bone marrow transplant and solid organ transplant units… renal, hepatic
- Medical Surgical services
- C. difficile infection
- Patients with enteric acquisition of AMR organisms: VRE, AmpC, ESBL, CRE, NDM, KPC

Microbe Therapies: *the to do list*

- ❑ Establish the scientific basis of antibiotic stewardship by quantification of the effect of antimicrobial chemotherapy on the host microflora….long overdue.
- ❑ Revise the formulary to optimize selectivity of treatment. We have agents we don't use often enough, use bad agents too frequently, carelessly, unknowingly.
- ❑ While low cost drugs might be narrow spectrum, that's not always the case. eg. Ceftriaxone is inexpensive.
- ❑ Probiotics need careful RCTs on larger scale to establish a benefit, yes or no. Many lab and animal studies suggesting protection.
- ❑ Out of the FMT studies, the keystone microbial groups / clusters should emerge that would confer colonization resistance.

Where damage to the protective indigenous microbiota is not preventable, where should we focus re-complementation of microbes ??

- Intensive care unit ecology, comparative trials on the benefits of maintaining or bolstering the normal microbiota components to prevent ventilator pneumonia, sepsis/bacteremia and multi-organ failure.
- Immunocompromised hosts, particularly leukemia / BMT and solid organ liver> renal transplants, where neutropenia, intestinal dominance by proteobacteria often AMR microbes, leads to translocation, bacteremia and death.
- General medical/surgical patients..more of these, but impact is lower, but persons discovered to persistently harbour AMR organisms
- [Obesity, Autism, NAFLD, IBD, IBS, C.difficile].

In this era of the emergence of pan-antibiotic resistant pathogens, protecting and where damage is done, re-complementing the normal microbiota / microbiome is the largest missing link in the health care.

HEALTH CARE SYSTEMS THEMSELVES NEED TO INVEST IN SELF-PROTECTION AND NOT DEPEND ON FUNDING AGENCIES TO DO THE JOB FOR THEM. FUNDING AGENCIES CAN BE CATALYSTS HOWEVER.