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In this supplement: Antimicrobial resistance and innovation

In this issue, read about how normal flora can now be manufactured to treat *Clostridium difficile* and potentially other conditions, learn how optimal vaccine use can minimize the need for antibiotics, and see how the Canadian Institutes of Health Research has been funding research on antimicrobial resistance (AMR) innovation. In the ID News section read about the use of nanotechnologies to treat HIV, tuberculosis and yeast infections, and learn about a new protein inhibitor to treat malaria. This is the last in a series of theme issues to highlight the three pillars of Canada's Federal Action Plan on AMR: Surveillance, Stewardship and Innovation. If we all continue to work together on this three-pillar approach, the potentially devastating effects of AMR can be averted.

Upcoming conferences

March 9–12, 2016: European Society of Clinical Microbiology and Infectious Diseases (ESCMID). International Meeting on Microbial Epidemiological Markers. Navigating Microbial Genomes: Insights from the Next Generation. Estoril, Portugal.
https://www.escmid.org/research_projects/escmid_conferences/immem_xi/

March 30-April 2, 2016: Association of Medical Microbiology and Infectious Disease Canada - AMMI Annual Conference. Vancouver, British Columbia
https://www.ammi.ca/annual-conference/

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Innovation

Using bugs as drugs: Microbial ecosystem therapeutics

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Abstract

The human gut harbours a dense and highly diverse microbial ecosystem—the microbiota—that plays an important role in the maintenance of health. Modern lifestyle practices, including widespread antibiotic use, have degraded microbiota diversity, compromising the integrity of this vital ecosystem and creating susceptibility to diseases such as *Clostridium difficile* infection. Treatment of patients to restore the diversity of the gut microbiota offers a logical solution to disease. Although fecal microbial therapy (FMT) has started to gain traction as an effective method to effect this restoration, it is not without risks and there are significant barriers to its implementation in the clinic. Some of the risks and challenges with FMT are addressed by microbial ecosystem therapeutics (MET), an alternative approach to FMT that uses selected, defined microbial ecosystems to redress microbiota balance and functionality. The time has come for the use of bugs as drugs.

Introduction

Human beings are colonized by trillions of microbes; every skin and mucosal surface houses a diverse community of microbial cells (1). The gut contains by far the greatest density and diversity of microbes in the human body: the colon contains up to 1,012 microbial cells per gram (2), a microbiota made up largely of bacteria, but also including smaller numbers of archaea (prokaryotes distinct from bacteria), yeasts and protists (eukaryotic microbes) (3). Although some opportunistic pathogens reside among this microbiota, the vast majority of species within the ecosystem are either benign, or more commonly are beneficial, carrying out a great many functions for us that aid in the maintenance of health. Such functions include modulation of the immune system, production of beneficial substrates such as butyrate (which acts as a food source for colonocytes, among other health-promoting attributes) and vitamins, and production of certain chemical signaling molecules that may play important roles in the control of pathogenic exposure to the host (4,5).

Despite this plethora of functions, modern medicine has, until fairly recently, ignored the importance of the gut microbiota, and in fact has fostered the use of antibiotics for treatment of infections. As well as encouraging antimicrobial resistance, widespread antibiotic use has inadvertently impaired the integrity of the gut microbiota through collateral damage (6). In fact, it has been theorized that such ecosystem damage and subsequent loss of microbial species diversity may be a major contributing factor towards the increase of several chronic diseases that currently plague modern society (7). Certainly this theory is supported by a growing weight of evidence demonstrating reduction of the diversity of the gut microbiota associated with many diseases from inflammatory bowel disease to psoriatic arthritis (8,9). However, because the average gut microbiota contains a large number of microbial species, and because the species profile of a given individual is distinct and unique (10), trying to define the underlying mechanisms of these diseases as they relate to the microbiota is challenging.

In this overview we describe how depletion of ecosystem diversity may lead to disease, using the example of *Clostridium difficile* infection (CDI). We also discuss how strategies to replace lost ecosystem diversity using fecal microbes can be useful in the treatment of CDI, and how the risks and challenges of this approach can be mitigated by the use of defined microbial ecosystems.

Treating recurrent *Clostridium difficile* infection

There is one condition that is clearly understood to be a result of gut ecosystem disturbance and lack of microbial diversity—this is CDI. A persistent problem in the hospital environment, CDI is usually a complication of broad spectrum antibiotic use, where these antibiotics reduce the diversity of the gut microbiota of the patient; with this protective diversity gone, *C. difficile* can grow unchecked, to high numbers (11). As well as antibiotic use, other interventions known to decrease gut microbial diversity, such as the use of proton pump inhibitors (PPIs), or chemotherapy, may also contribute to the risk of developing CDI (12−14). Once a particular growth threshold has been exceeded, *C. difficile* produces a range of exotoxins with damaging effects on human colonocytes, leading to profuse diarrhea that can progress to pseudomembranous colitis, toxic megacolon and, in severe cases, death (11).

The current standard therapeutic solution to this problem is to apply more antibiotics. Drugs such as metronidazole or oral vancomycin are used, which have activity against *C. difficile*, to reduce the numbers of the pathogen and hence the toxigenic load (15). However, there is some backwards logic here; the CDI patient suffers from a lack of gut microbial diversity, usually as a result of antibiotic use. Further antibiotic use may have a short-term benefit as the numbers of the pathogen may be temporarily reduced, but *C. difficile* is an endospore-forming bacterium that can sporulate when faced with an inhospitable environment (such as the presence of antibiotics). The spores are resistant to metronidazole and oral vancomycin, allowing *C. difficile* to germinate and cause problems once more when the antibiotic is removed (16). Meanwhile, antibiotic exposure removes the natural resistance to *C. difficile* colonization—the gut microbiota. CDI patients increasingly get caught up in a cycle of *C. difficile*-suppressive antibiotic use, which has negative effects on the protective gut microbiota, preventing recovery from infection and leading to recurrent CDI. This problem is a big one—a recent survey across the United States carried out in 2011 estimated half a million cases of CDI for that year (17). Recurrent CDI accounts for 10% to 20% of cases (18).

An alternative to antibiotic treatment for recurrent CDI lies in restitution of the diversity of the gut microbiota. Re-establishing this protective diversity displaces *C. difficile*, rather like how re-seeding a damaged lawn with healthy new turf can displace the weeds. At its most primitive, this is achieved through use of "fecal microbial therapy" (FMT), literally a transfer of stool from a healthy donor into a patient by way of rectal enema, colonoscopy, or even nasoduodenal tube (19). The approach is not new; practitioners of Chinese medicine used preparations of fecal microbes to treat diseases such as dysentery as early as the 4th century BC, and veterinarians have used the principle of fecal transplant to treat animals with gastrointestinal problems for decades. Whilst the practice is unpleasant, it is clearly effective; 81% of patients provided FMT for recurrent CDI were rapidly cured of their infections with one dose of FMT, compared to 31% given vancomycin in a recent clinical trial (20). However, FMT is not without risk; although when done under medical supervision donors are rigorously screened for known pathogens potentially passed on through stool, there is so much diversity within the human gut microbiota, with many as yet uncharacterized microbial species, there is no way to currently know whether unknown pathogens are being transferred by the practice.

Challenges with FMT

Although FMT is a promising alternative to antibiotic use for treatment of recurrent CDI, it is difficult to regulate. Regulation is problematic because each donor's stool can be considered as a different, complex therapeutic (21). As yet there is no current consensus on how to define and standardize the treatment, although the use of frozen stool products and "super donors" goes some way to address the issue (19). A further complication is that government-sanctioned administrative oversight of the procedure may limit its availability and therefore has the potential to drive the practice underground; FMT can be performed at home

with no medical supervision, using materials that can be easily procured from a drug store. Recently, Health Canada produced a guidance document *Regulation of Fecal Microbiota Therapy for the Treatment of C. difficile Infections* to inform FMT practitioners of the government stance on the practice of FMT (22); Health Canada views stool used therapeutically as a biologic drug, because it has been derived from a human source (21).

There are also concerns about the long-term safety of FMT. While for recurrent CDI the short-term benefits are clear, so far there has been a dearth of studies of the long-term effects of the treatment. And while such negative effects may be negligible for elderly patients, CDI is becoming increasingly common in younger, otherwise healthy people (23). So, how should we proceed with this promising therapy, while also ensuring safety?

Microbial ecosystem therapeutics

The challenges of FMT can be addressed by the development of standardized "microbial ecosystem therapeutics" (MET). MET can be thought of as a new type of "probiotic," one in which beneficial microbes are carefully selected from a single healthy donor, thoroughly screened for any potential detrimental attributes (e.g., antibiotic resistance) and then recombined into an ecosystem. Microbes within ecosystems tend to work together synergistically, and we hypothesize that our approach capitalizes on this microbial synergism, enhancing any beneficial effects. Our prototype product, MET-1 (RePOOPulate), which was shown to be effective as treatment for recurrent CDI in a small proof-of-principle trial, was created through careful culture of the diversity from the stool of a healthy woman (24). From this pool of isolates, a subset of 33 strains was selected, each with minimal antibiotic resistance and lacking any known virulence determinants; these were formulated into an ecosystem and tested for functional integrity in vitro using chemostat technology.

The resulting, defined microbial ecosystem has many advantages over FMT as a therapeutic, including the ability to standardize the manufacturing process and to create a quality controlled product, one of the key guidelines for regulation of a biologic drug. This product can potentially be delivered orally as a live, freeze-dried preparation, simplifying treatment. Perhaps the greatest advantage of this approach over the use of stool is the fact that the long-term effects of the treatment may be more easily studied such that, as with any biologic drug, safety of the product can be increasingly ensured over time. MET-1 is the first in a series of MET products currently being created to provide treatment options tailored to patient lifestyle—for example, diet, known to be a key driver of ecosystem diversity (25). While the regulatory hurdles to bring these products to market are high, the expected benefits are higher, and the ability to track introduced microbes in a patient may also help to answer key ecological questions about, for example, the influence of the host on ecosystem function and stability. However, it is important to understand that development of the MET principle is still in its infancy and there are many questions that still need to be answered—for example, what are the essential components of MET ecosystems that allow for their therapeutic benefit? How important is functional redundancy of a MET ecosystem to its efficacy in the treatment of disease? Do introduced MET microbes colonize the host indefinitely, or do they simply act as a "band aid" to allow recovery of the recipient's original ecosystem? Are there any detrimental effects of MET in comparison to FMT?

Conclusion

We now have the scientific basis to use bugs as drugs. FMT has documented benefit, but it also has its challenges. We believe that MET represents a new chapter in medicine. In this new era, microbes will finally be considered as our allies, and their properties, such as their antivirulence determinants, may be leveraged for the preservation of health.

In the future we should consider the use of MET in other diseases where adverse compositional changes in the gut microbiota may be a key factor in disease. There are indications in the literature, for example, that FMT-based approaches may have an impact in such diseases as ulcerative colitis, obesity and

metabolic syndrome (8,26-29). What is needed is the introduction of tailored METs developed to address the underlying gut microbial dysbiosis in these diseases, and to monitor the outcomes of MET-based intervention in well-designed clinical trials.

Conflict of interest

The authors are co-founders of Nubyota LLC, a company created to commercialize the application of microbial ecosystem therapeutics in medicine.

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Commentary

Immunization as a tool to combat antimicrobial resistance

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Abstract

Vaccines and immunization programs can play a key role in addressing the growing challenge of antimicrobial resistance (AMR). Amongst the high priority vaccines in development are several AMR pathogens, including: *Clostridium difficile, Staphylococcus aureus, Streptococcus pneumoniae, Mycobacterium tuberculosis* and *Neisseria gonorrhoeae*. There is evidence that vaccination can reduce the prevalence of AMR microbes, as demonstrated by both pneumococcal and *Haemophilus influenza* b vaccines. Research continues on many vaccine-preventable diseases, many of these AMR pathogens, including HIV and universal influenza vaccines. Not only do vaccines prevent infections, they can also prevent secondary opportunistic infections from AMR microbes—for example, bacterial pneumonia following influenza infections. The reduced need to treat these opportunistic infections would also mitigate the advance of AMR microbes in our communities. However, vaccines are not a panacea. One downside to the use of vaccines to address AMR is vaccine hesitancy, which undermines efforts to achieve herd immunity, but this is being increasingly addressed by public health education campaigns.

Introduction

Is the 21st century going to be remembered as the beginning of the post-antibiotic era? With each passing year, our list of effective antimicrobials is slowly shrinking, as more microbes become resistant in both the human and animal settings. At the same time, fewer antimicrobials are being brought to the market. Microbes by their nature continually adapt to survive the antimicrobial treatments we use to combat them, resulting in an ever increasing level of antimicrobial resistance (AMR).

The rate of methicillin-resistant *Staphylococcus aureus* (MRSA) infections among hospitalized patients in Canada increased nine-fold from 1995 to 2009 (0.4 - 3.8 per 10,000 patient days) (1-3). With concerted efforts, rates have been slowly decreasing but in 2014 they were still reported at 2.8 per 10,000 patient days (2). In 2012, 33% of MRSA infections identified in hospitalized patients were acquired in the community, compared to 17% in 1995 (3). In northern Canadian communities, community-associated MRSA infection rates have been higher than anywhere else in North America (4).

Each year in Canada, more than 18,000 hospitalized patients acquire infections that are resistant to antimicrobials. The incidence rates, though relatively low for vancomycin-resistant Enterococci (VRE) infections in Canada, have slightly increased from 2009 to 2013 (0.31 and 0.52 per 10,000 patient days, respectively) (2). Deaths directly and indirectly related to *Clostridium difficile* infection (attributable mortality) alone increased three-fold from 1.5% to 5.7% between 1997 and 2005, respectively (5-7); recent efforts have kept this relatively stable from 2011-2014 (2).

The prevalence of microbes with resistance to more than one antimicrobial compound and our general lack of new antimicrobial compounds have heightened the concern on how to manage AMR in the future. As an immediate response, we urgently have to look for new antimicrobials, but also consider other control measures, like the use of vaccination to develop herd immunity against the microbes that have developed AMR.

The purpose of this article is to highlight the role that vaccines and immunization programs could play in the battle against AMR pathogens.

The case for vaccines

Vaccines are undoubtedly one of the best investments in health. Immunization programs have contributed enormously to reducing the burden of infectious diseases, and are responsible for much of the falling rates of morbidity and mortality worldwide. In December 2010, global health leaders committed to making the next 10 years the Decade of Vaccines—to ensure discovery, development and delivery of lifesaving vaccines globally, especially to the poorest countries. Innovative vaccine approaches are under development and being prioritized to include some of the most significant threats posed by AMR, through the development of a Canadian Action Plan on Vaccine Research, Innovation and Development, the Global Vaccine Action Plan (8), and many other international efforts (9) to reduce the impact of vaccine-preventable diseases. Amongst the high priority vaccines in the research and development pipelines are several AMR pathogens, including: *Clostridium difficile, Staphylococcus aureus, Streptococcus pneumoniae, Mycobacterium tuberculosis, Neisseria gonorrhoeae*, HIV, and influenza.

Examples of success

There is evidence that vaccination can reduce the prevalence of AMR microbes. In South Africa, a 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2009 and replaced with a 13-valent vaccine (PCV13) in 2011. In 2012, it was estimated that 81% of 12-month-old children had received three doses of the vaccine. Prior to vaccination, 83% of multidrug-resistant pneumococcal disease isolates were serotypes contained in PCV7. From 2009 to 2012, amongst children younger than 2 years of age, the rate of invasive pneumococcal disease caused by penicillin-resistant isolates declined by 82%, ceftriaxone-resistant isolates by 85%, and multidrug-resistant variants by 84% (10). The pneumococcal vaccine programs in children have had an additional impact on the capsular types infecting adults, thus a broader range of impact than just the targeted age group.

Before the Hib vaccine was developed, up to 30% of *Haemophilus influenzae* type b (Hib) bacteria, which used to be a common cause of meningitis in young children, had become resistant to amoxicillin, a commonly used antibiotic. Since the introduction of Hib vaccines, the number of *Haemophilus influenza* type b infections caused by drug-sensitive and drug-resistant bacteria has decreased by more than 96% in infants and children.

Vaccines not only help to prevent a targeted infection, they may prevent associated opportunistic infections as well. The effect of immunization on the morbidity and mortality associated with opportunistic infections—many of these AMR microbes—has a considerable impact on public health, for example, the prevention of bacterial pneumonia following influenza infections. The reduced need to treat for these opportunistic infections also has a direct impact on the further development of multidrug resistant AMR microbes and increased incidence of AMR microbes in our communities.

New and better vaccines

A safe and effective prophylactic or therapeutic HIV vaccine could dramatically change the lives of those susceptible to this virus and alleviate the economic burden associated with HIV infections worldwide. Microbes by their nature are very adaptable to survival challenges, whether that be avoiding the host's immunological responses or evolving rapid resistance to antimicrobials. The history of HIV and the development of drug resistance to successive iterations of new drugs are legendary. However, the use of drug cocktails and tailoring treatment regimens to individuals have helped increase the lifespan of those infected from the bleak days of the 1980s to today. That being said, daily drug treatment is a demanding regime and not without adverse drug effects. Non-adherence to this regime can lead to the development of drug resistance. The pursuit of a safe and effective HIV vaccine has now been explored by many excellent research groups for close to 30 years, but as yet no highly effective vaccine exists. Hence, for some infectious diseases, vaccines may not be the most effective strategy in the short term and should not be seen as the sole response to AMR development.

Until a universal influenza vaccine is developed, every year we have to prepare for a new influenza season, with a new combination of influenza A and B vaccines. Not only is genetic drift and shift an ever-looming problem, but resistance to the antivirals used to treat influenza infections can develop. Fortunately, most influenza A and B virus strains are susceptible to the neuraminidase inhibitor antiviral medications, oseltamivir, zanamivir and peramivir (11,12). However, sporadic oseltamivir-resistant 2009 H1N1 virus infections have been identified, including with rare episodes of limited transmission (13-17), but the public health impact has been limited to date. During the 2013−2014 season, 98.2% of the 2009 H1N1 viruses tested for surveillance were susceptible to oseltamivir, and 100% of the 2009 H1N1 viruses tested were susceptible to zanamivir. Additional sporadic cases of oseltamivir-resistant 2009 H1N1 virus infection can be expected, and ongoing surveillance for oseltamivir resistance among influenza viruses is essential for public health since oseltamivir is the most widely used antiviral medication. Though combination therapy approaches can be used, new antivirals and other approaches to combating influenza are needed. The seasonal and pandemic vaccine programs can prevent influenza virus infections, dramatically reducing the likelihood of developing antiviral resistance. However, the current vaccines have variable vaccine efficacy, as we are witnessing in the 2014−2015 influenza season in Canada, requiring much more research and development to enhance their effectiveness, especially in the very young and the elderly. The use of higher vaccine doses and the addition of new adjuvants to existing vaccines may increase vaccine efficacy, but the "Holy Grail" would be the development of universal influenza vaccines which would protect against influenza in the face of virus drift and possibly even virus shifts that occur over time. These are being explored by several companies and research groups at this time, but this virus remains a global public health challenge.

Challenges

Ironically, in the very countries that have benefited the most from national immunization programs, there has been a loss of public confidence in vaccines by a small minority of people. Personal decision making related to vaccine acceptance is not driven by scientific nor economic arguments, but by a mix of psychological, sociocultural and political factors, all of which need to be understood and taken into account by policy and other decision makers. Public trust in vaccines is very complex and building trust depends on understanding vaccine perception and vaccine risks, historical experiences, religious or political affiliations, and socioeconomic status. Although providing accurate, scientifically based evidence on the risk-benefit ratios of vaccines is important, it is not enough to address the growing gap between current levels of public confidence in vaccines and levels of trust needed to ensure adequate and sustained vaccine coverage and long-lasting herd immunity. The recent measles outbreaks in North America and Europe in many whom have not been vaccinated by choice has highlighted the vaccine hesitancy issue. Further research into all aspects of vaccine hesitancy will help inform decision making on immunization and its importance in the fight against infectious diseases, including AMR microbes.

Conclusion

Vaccines have been one of the best public health tools in our fight against the impact of infectious diseases worldwide. Vaccines have played a paramount role in the eradication of smallpox and in bringing polio and measles close to elimination. Could vaccines show a similar global public health impact on AMR microbes? Government, industry, academia and non-governmental organizations worldwide are engaging more and more in the battle against AMR microbes. Though the pursuit of new effective antibiotics is crucial in this fight, other treatment and preventive approaches are needed to prevent the spread of AMR microbes. Development of new and improved vaccines may be crucial in our fight against AMR microbes and for maintaining and enhancing public health worldwide. However, vaccines can only be effective if the populations they are designed to protect agree to be vaccinated in the numbers required to induce herd immunity. Addressing the issue of vaccine hesitancy, through active education campaigns and other approaches, is crucial in the ongoing fight against all vaccine-preventable diseases including those that have developed resistance to our antimicrobial armada.

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Conflict of interest

None

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Overview

The Canadian Institutes of Health Research response to antimicrobial resistance

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Abstract

Antimicrobial resistance (AMR) has been a research priority for the Canadian Institutes of Health Research (CIHR), Institute of Infection and Immunity (III) since its inception, and a number of strategic research initiatives have been launched to address this global health problem by promoting and supporting research related to mechanisms and processes that impact the emergence and spread of resistance among individuals and within the environment. Here we will present research initiatives on AMR led by CIHR-III, which include national programs as well as international partnerships with the United Kingdom and the European Union, in addition to interesting outcomes of these initiatives.

Introduction

The Canadian Institutes of Health Research (CIHR) is the Government of Canada's health research investment agency. The Institute of Infection and Immunity (III) is one of the thirteen "virtual" institutes of CIHR which supports research and builds research capacity in the areas of infectious diseases and immunity in an integrated approach based on four broad themes (bio-medical research; clinical research; research on health systems services; and research on social, cultural, environmental and population health). Based on its 2013−2018 Strategic Plan (1), CIHR-III has developed a number of strategic research initiatives to stimulate targeted areas, including antimicrobial resistance (AMR).

CIHR investments in AMR research and the Federal Action Plan on AMR

AMR is a growing public health threat that involves interplay between multiple sectors. The loss of effective antimicrobials is reducing the ability to prevent and treat infectious diseases, while also impacting our health care system, global trade, and the agriculture, environment, and health sectors. The World Health Organization has now included AMR as one of the most pressing public health issues. In October 2014, the Government of Canada released *Antimicrobial Resistance and Use in Canada: A Federal Framework for Action* (2) to serve as a starting point for cohesive engagement and mobilization of all who are accountable for action on AMR and antimicrobial use. Efforts are being focused on three priority areas: Surveillance, Stewardship and Innovation. Through its open and strategic programs, CIHR invested $93.8 million in AMR research between 2009-2010 and 2013-2014, including over $15 million in 2013−2014 alone. Most of the funding has been geared toward innovation; moving forward these programs can help to better identify strategies/approaches for stewardship or surveillance.

CIHR-III's strategic research initiatives on AMR

Antimicrobial resistance, health system implications and health outcomes

During consultation with the research community for our CIHR-III 2013−2018 Strategic Plan, AMR was clearly identified as a critical strategic research priority. This represents a continuum for CIHR-III, as the Institute has invested in AMR research since its inception. Earlier investments included an Antimicrobial Resistance, Health System Implications and Health Outcomes request for applications resulting in two projects funded. The first funded project was on Antimicrobial Use and Resistance in Seniors and

studied methicillin-resistant *Staphylococcus aureus* (MRSA) incidence in elderly hospitalized patients. The team showed that the epidemiology and clinical features of those patients (being more likely to be colonized by MRSA) are different from younger patients (3). The second project, entitled Community-Acquired Antimicrobial Resistant Bacteria in Northern Canadian Communities, led to the creation of the Northern Antibiotic Resistance Partnership (NARP), a team composed of community members, health care professionals, educators and research scientists studying resistant bacteria in northern communities (4). The group has established a surveillance program to monitor bacterial infections and antibiotic use, has developed educational tools for health care providers and the general population, and has produced a community-associated case control study to determine the risk factors associated with community-acquired MRSA (CA-MRSA) infections in northern regions of Saskatchewan.

Safe Food and Water Research Initiative

The Safe Food and Water Research Initiative, which was sparked in part by the Walkerton tragedy, brought together governmental and private sector organizations in order to develop a national research strategy to prevent and respond to diseases caused by foodborne and/or waterborne pathogens or their toxins. The two requests for applications launched resulted in the funding of four projects related to AMR—a $2.8 million investment in total. Outcomes of this investment include the establishment of a baseline of resistance profiles and the mechanisms of resistance observed in *Campylobacter jejuni* in poultry from Alberta (5), and the characterization of cefotoxin-resistant *Escherichia coli* strains from Canadian water sources, as well as prevention measures to avoid bacterial contamination of water streams (6,7).

Novel Alternatives to Antibiotics Initiative

The Novel Alternatives to Antibiotics Initiative was designed to enhance the existing funding available through the CIHR open competitions by attracting applications focused on novel approaches to antibiotic resistance in which Canada had little or no research capacity. Three priority themes were determined: immune systems; phage therapy (or the use of viruses to infect and kill pathogenic bacteria); and physical systems and biomaterials. This investment was the product of the collaboration of several public and private sector partners and the funded projects resulted in several interesting outcomes, including promising results using a non-invasive phage delivery technique through aerosol to treat respiratory tract infection (8) and the identification of new antibiotics against MRSA. Several patents have been filed and intellectual property has been transferred to a Canadian spin-off company following the latter project (9).

The Canada/U.K. partnership on antimicrobial resistance

Since 2007, Canada has partnered with the Medical Research Council in the United Kingdom, resulting in several workshops and joint competitions. Following an initial workshop and catalyst funding, in September 2010, a team grant involving a partnership between Canada and the U.K. on antibiotic resistance was launched that built on existing collaborations between the two countries and provided four years of support. The first team funded dealt with bacterial cell wall synthesis and is in the process of testing several potential inhibitors with the aim of identifying leads for novel antibiotics (10-12). The second team studied bacterial resistance to β-lactam antibiotics which led to multiple outcomes, including the design, synthesis and testing of new candidate inhibitors of metallo-β-lactamases, and the characterization of inhibition of key metallo β-lactamases targets by known compounds (13,14). The Canada/U.K. partnership investment was close to $4 million and allowed the funded teams to secure additional funding and partnerships as well as building capacity in both areas. A second workshop, on Translational Strategies to Combat Antibiotic Resistance, followed in 2013 and provided recommendations for increasing awareness, supporting public and private sector partnerships, increasing financial investments and supporting collaborative approaches (15).

Joint Programming Initiative on Antimicrobial Resistance

Canada, through CIHR, is a major funder of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), an international network of 20 countries providing a collaborative platform to take AMR from awareness to action by supporting research and facilitating its translation to industry and policy. The aim of this initiative is to develop integrated approaches to pursue unique world-class research on AMR. Through its Strategic Research Agenda, JPIAMR enhances multidisciplinary collaboration and ensures that knowledge gaps are quickly identified and filled. Actions within six priority topics (therapeutics, diagnostics,

surveillance, transmission, environment, and interventions) will be translated into new prevention and intervention strategies to improve public health and deliver economic and societal benefits. Launched in 2014, the transnational call InnovaResistance: Innovative Approaches to Address Antibacterial Resistance resulted in the funding of seven consortia, six of which include Canadian researchers (16). The primary aim of this joint call is to combine the resources, infrastructures and research strengths of multiple countries in order to overcome antibiotic resistance. The call focused on the re-evaluation of existing antimicrobial compounds (alone or in combination with other drugs, immune-modulators or antibacterial approaches), identification of new bacterial targets and/or therapeutic compounds, discovery of novel therapies to overcome known antimicrobial resistance mechanisms and restore susceptibility to conventional antibiotics, or drug combinations and strategies to inhibit or reduce the acquisition of resistance. The Canadian nominated projects were in line with several of those priorities.

Recently JPIAMR announced another transnational call to be launched in early 2016 in which 20 funding organizations throughout the world will take part, including Canada. The topic of that call will promote research to "unravel the dynamics of transmission and selection of antimicrobial resistance (AMR) at genetic, bacterial, animal, human, societal, and environmental levels, in order to design and evaluate preventive and intervening measures for controlling resistance" (17).

JPIAMR is dedicated to the One Health approach where interdisciplinary collaborations are created to tackle health care issues. Hence, investigation of the mechanisms leading to the spread of resistance in and between different reservoirs, including animals, the environment and people, will contribute to the design of preventive measures to address the public health threat of AMR.

Other CIHR strategic investments

Related to the One Health approach, CIHR has developed the Environments and Health Signature Initiative which integrates a nexus approach (three nexus areas) through the study of cumulative environmental exposures, and their interconnections, intersections and impacts on health and disease across the life course (18). The JPIAMR transnational call to come in 2016 aligns with the topics of the Environments and Health Signature Initiative. Both will support research to examine how intersectoral strategies and approaches contribute to improvements in population health and health equity.

Conclusion

Antimicrobial resistance is a serious problem that causes the hospitalization of more than 250,000 Canadians every year, and more than 18,000 hospitalized patients acquire drug-resistant infections (19). For instance, deaths directly related to *Clostridium difficile* alone have increased five-fold in the past decade (20). It is also a severe economic drain. Initial research has shown that if no action is taken, a continued rise of resistance would lead, in 2050, to 10 million deaths and a cost of $100 trillion USD worldwide (21). CIHR-III is committed to improving the lives of Canadians and the global community by supporting innovative research in AMR. In the past five years, CIHR and III have invested more than $93.8 million in combatting AMR. AMR research will remain a focus as the threat continues to expand. CIHR and III play a proactive role in the Federal Action Plan on AMR and will continue to promote innovation by supporting national and international collaborations and multi-theme initiatives in order to allow leveraging of investments. Future strategic research investments will deal with both the development of novel molecules but also with strategies to protect the current antibiotics available through better diagnostics and stewardship. There is currently momentum in the research field as well as in the policy arena to find innovative solutions for tackling the vexing problem of AMR, which has been coined by the 2013 World Economic Forum as the greatest risk of hubris to human health.

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Conflict of interest

None

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ID News: Innovation and antimicrobial resistance

Roy U, Barber P, Tse-Dinh Y-C, Batrakova EV, Mondal D, Nair M. **Role of MRP transporters in regulating antimicrobial drug inefficacy and oxidative stress-induced pathogenesis during HIV-1 and TB infections.** *Front. Microbiol. 2015 6:948. doi: 10.3389/fmicb.2015.00948*

Multi-Drug Resistance Proteins (MRPs) are membrane transporters known to regulate the efficacy of a broad range of anti-retroviral drugs (ARV) used in highly active antiretroviral therapy (HAART) and antibacterial agents used in Tuberculus Bacilli (TB) therapy. MRPs can also regulate cellular oxidative stress, which may contribute to both HIV and/or TB pathogenesis. Known members of the MRP family in HIV infected cells exposed to ARV drugs have a role in drug-inefficacy. Currently, nine members of the MRP family (MRP1-MRP9) have been identified (and may play a role in) precipitating cellular dysfunctions manifested in these chronic infectious diseases. We also provide an overview of different novel experimental strategies that are being utilized to overcome the drug resistance and disease pathogenesis mediated by these membrane transporters. (For example) following antiretroviral therapy, HIV persists in the long-lived cells of the central nervous system (CNS) as a latent infection. Most antiretroviral drugs cannot reach these sites in therapeutic doses, because they are either unable to penetrate the CNS, or they are excluded by efflux transporters in the blood brain barrier. Rates of HIV associated neurocognitive impairment will likely rise in the coming years as anti-HIV therapies continue to extend the lifespan of patients. Eliminating CNS reservoirs of HIV will greatly increase the quality of life and lifespan of seropositive individuals. Engineered nanoparticles may provide the ability to bypass the blood brain barrier and reach these HIV reservoir sites. Previous studies have indicated that polymeric nanoparticles loaded with HAART drugs can cross blood brain barrier and reach the CNS reservoir at therapeutic levels in animal models.

Artunduaga Bonilla JJ, Paredes Guerrero DJ, Sánchez Suárez CI, Ortiz López CC, Torres Sáez RG. **In vitro antifungal activity of silver nanoparticles against fluconazole-resistant Candida species.** World J Microbiol Biotechnol. 2015 Nov;31(11):1801-9. doi: 10.1007/s11274-015-1933-z. Epub 2015 Sep 3.

Current advances in nanotechnology, such as silver nanoparticles (AgNPs), constitute a promising alternative in the development of new antimicrobial agents. In this study, AgNPs were synthesized by eco-friendly method, using cysteine as a reducing agent. Also, antifungal activity against Candida species with resistance to fluconazole was evaluated through determination of Minimum Inhibitory Concentration (MIC50) according to protocol M27-A3 of Clinical and Laboratory Standards Institute (CLSI) and Minimum Fungicide Concentration (MFC). This study was carried out with strains Candida krusei and Candida glabrata. As a result, the formation of spherical nanoparticles was obtained with mean sizes of 19 nm and positive surface charge. Values of MIC50 were 0.1 µg ml(-1) AgNPs for the studied species, and MFC were 0.25 and 0.5 µg ml(-1) for C. glabrata and C. krusei, respectively. The AgNPs synthesized showed cytotoxic effect in 50% of Murine Fibroblast Cells (CC50) at a mean concentration of 10 µg ml(-1) (100 times higher than MIC50). Consequently, AgNPs could be considered as an alternative potential in the development of new antifungal agents with minimum cytotoxicity in fibroblasts and lethal action on Candida species with resistance to conventional antifungal compounds.

Baragaña B[1], Hallyburton I, Lee MC, Norcross NR, Grimaldi R, Otto TD. **A novel multiple-stage antimalarial agent that inhibits protein synthesis**. Nature. 2015 Jun 18;522(7556):315-20. doi: 10.1038/nature14451

DDD107498 is a compound with a potent and novel spectrum of antimalarial activity against multiple life cycle stages of the Plasmodium parasite, with good pharmacokinetic properties and an acceptable safety profile. DDD107498 demonstrates potential to address a variety of clinical needs, including single-dose treatment, transmission blocking and chemoprotection. DDD107498 was developed from a screening programme against blood-stage malaria parasites; its molecular target has been identified as translation elongation factor 2 (eEF2), which is responsible for the GTP-dependent translocation of the ribosome along messenger RNA, and is essential for protein synthesis. This discovery of eEF2 as a viable antimalarial drug target opens up new possibilities for drug discovery.