Microbiology AUSTRALIA

OFFICIAL JOURNAL OF THE AUSTRALIAN SOCIETY FOR MICROBIOLOGY INC.

Volume 37 Number 4 November 2016

A special issue in association with The Microbiology Society

Microbial diseases of travel

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References: 1. DIFICID Australian Approved Product Information, June 2015. **2.** Cornely OA *et al. Lancet* 2012; 2(4): 281–89. **3.** Louie TJ *et al. N Engl J Med* 2011; 364: 422–31.

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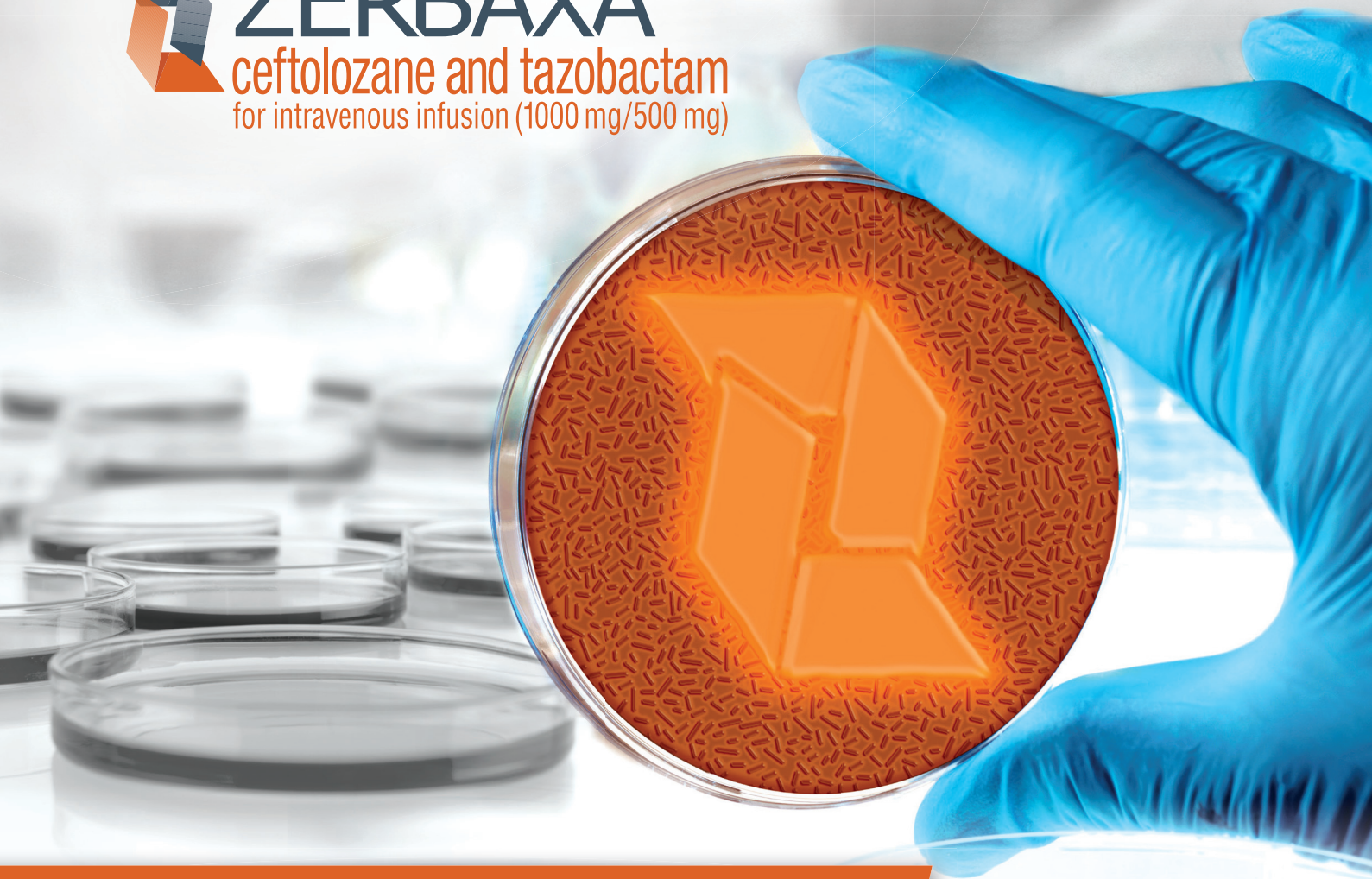

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References: 1. ZERBAXA Approved Product Information, 4 November 2015. **2.** Sanford Guide (Web Edition) 2016. Available at: http://webedition.sanfordguide.com/ **3.** Zhanel GG, Chung P, Adam H, Zelenitsky S, Denisuik A et al. 2014. Ceftolozane/Tazobactam: A Novel Cephalosporin/ß-Lactamase Inhibitor Combination with Activity Against Multidrug-Resistant Gram-Negative Bacilli. *Drugs*, 74(1):31–51. **4.** Solomkin J, Hershberger E, Miller B, Popejoy M, Friedland I et al. 2015. Ceftolozane/Tazobactam Plus Metronidazole for Complicated Intra-abdominal Infections in an Era of Multidrug Resistance: Results From a Randomized, Double-Blind, Phase 3 Trial (ASPECT-cIAI). *Clin Infect Dis*, 60(10):1462–71. **5** Wagenlehner F, Umeh O, Steenbergen J, Yuan G, Darouiche R. 2015. Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract infections, including pyelonephritis: a randomised, double-blind, phase 3 trial (ASPECT-cUTI) *Lancet*, 385(9981):1949–56.

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Contents

Cover image: Courtesy of Stela Di (Pixabay).

Roy Robins-Browne
President of ASM

When I, a fresh young medical graduate, announced my decision to specialise in pathology my senior colleagues were impressed, because pathology is a 9-to-5 job that pays well. However, when I added that I was planning to sub-specialise in microbiology, they questioned my sanity. Didn't I know that infectious diseases had been conquered by the twin forces of immunisation and antimicrobials?

Fast forward 40 years to the present, and we see that infectious diseases remain amongst the most important causes of morbidity and mortality in humans and animals, and that resistance to antimicrobials is a major health concern. The problem of resistance is so dire that in September this year, the General Assembly of the United Nations saw fit to hold a high-level meeting to highlight the problems posed by antimicrobial resistance and to express support for the World Health Organization's blueprint to tackle this problem (http://www.un.org/pga/71/wp-content/uploads/sites/40/2016/09/Draft-AMR-Declaration.pdf). This was only the fourth time the UN General Assembly has met to address a health issue, a fact that underscores its seriousness.

This issue of *Microbiology Australia* is the result of a timely and successful collaboration between the ASM and our UK sibling: The Microbiology Society. Several articles cogently illustrate the continued importance of infectious diseases, exemplified by emerging and re-emerging infections, antimicrobial resistance, and the globalisation of infections through travel and trade. The remarkable malleability and adaptation of infectious agents that are illuminated by these articles will ensure that infections continue to be a major field of medicine and health research for many years to come.

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Microbial diseases of travel

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The November 2016 special issue of the *Microbiology Australia* is the first joint one with the Microbiology Society of the UK. Deciding on an appropriate theme for this issue, the 'Microbial Diseases of Travel' was a relatively straightforward task and a direct 'fallout' from the geographical distance that separates our two societies; In the recorded history of mankind, travel has been one of the most effective means of disseminating infectious diseases throughout and among different populations. Explorers carried with them, many infectious agents such as influenza, measles, small pox, typhus and yellow fewer resulting in devastating consequences for the indigenous populations that they encountered on their travels. Nowadays, with the current explosive rates and speed of travel the consequences of carrying infectious agents continue to be significantly detrimental to human, animal and crop populations even with our understanding of effective public health measures. Exposure to disease causing agents carried on wild animals can also be a potent force in the emergence of disease on travellers' return to their home country. In addition, migratory animals and birds can bring disease into far away countries as illustrated by the avian influenza.

Climate and environmental changes can also lead to the emergence of microbial diseases, which may not have been seen at a particular geographical location previously. Moreover, diseases such as brucellosis, HIV/AIDS, leishmaniosis and TB are known to have prolonged and variable incubation periods. As a result their clinical manifestations may appear long after the return from travel. As a direct consequence, it does not take a lot of imagination to see that tracking the source of infection can be difficult in some cases.

Due to the 'abrupt and dramatic changes in environmental conditions' such as changes in the altitude, temperature and humidity, travellers might also become more prone to diseases. The most commonly encountered microbial-mediated disease affecting travellers is called 'travellers' diarrhoea' and can be caused by many different foodborne and waterborne infectious agents. Prolonged shedding of infectious agents via the faecal-oral route can also happen, resulting in dissemination of the infectious agents along the travel path. Understanding the modes of transmission and corresponding general precautions can reduce the risks of infections. WHO lists these factors as:

- Foodborne and waterborne diseases
- Vector-borne diseases
- Zoonoses (diseases transmitted by animals)
- Sexually transmitted diseases
- Blood-borne diseases
- Airborne diseases
- Diseases transmitted via soil

At the destination or along the travel path, the risk of becoming infected depends on the sanitary and preventative measures taken, including vaccination. However, there are still some infectious diseases including some deadly ones, spread via travel, that have not generated effective vaccination programmes. A list with some of the diseases and causative agents associated with travel, where no vaccine are currently available is highlighted in Table 1.

The WHO uses the following criteria for classifying specific infectious diseases that involve potential health risks for travellers

- diseases that have a sufficiently high global or regional prevalence to constitute a significant risk for travellers;
- diseases that are severe and life-threatening, even though the risk of exposure may be low for most travellers;
- diseases for which the perceived risk may be much greater than the real risk, and which may therefore cause anxiety to travellers;
- diseases that involve a public health risk due to transmission of infection to others by the infected traveller.

The mode of travel can also be another factor in the increase or downgrade of infection risk. Most modern aircraft are fitted with high-efficiency particulate air (HEPA) filters and ventilation rates are controlled to recycle cabin air so that its quality is ensured. Well maintained HEPA filters trap dust particles and are adept at trapping bacteria and fungi. Transmission of infectious agents may occur between closely sitting passengers, as a result of personal

Table 1. Diseases and causative agents commonly encountered by travellers.

Disease	Causative agents
Amoebiasis	Parasitic amoeba
Angiostrongyliasis	Parasite
Anthrax	Bacterium
Brucellosis	Bacterium
Chikungunya	Virus
Dengue fever	Virus
Giardiasis	Parasite
Haemorrhagic fevers	Virus
Hepatitis C	Virus
Hepatitis E	Virus
Histoplasmosis	Fungus
HIV/AIDS	Virus
Legionellosis	Bacterium
Leishmaniosis	Parasite
Leptospirosis	Bacterium
Listeriosis	Bacterium
Lyme borreliosis (lyme disease)	Bacterium
Lymphatic filariasis	Parasite
Malaria	Parasite
Onchocereciasis	Parasite
The Plague	Bacterium
SARS (severe acute respiratory syndrome)	Virus
Schistosomiasis (Bilharziasis)	Parasite
Trypanosomiasis	Parasite
Typhus fever	Bacterium
Zoonotic influenza	Virus

hygiene decisions and shared fomites. If infectious diseases are to be avoided then the best advice is to strictly adhere to safety precautions and committed personal hygiene practices. An example is the transmission of Tuberculosis in air travel and the preventative measures are highlighted in the WHO Guidelines for Prevention and Control.

It is not just air travel that can spread disease: sea travel is also an effective 'transmission' environment with gastrointestinal disease outbreaks from contaminated food or water, norovirus infections, legionellosis, varicella and rubella all being reported.

Another concerning risk might derive from poorly stored seafood under unchilled conditions (e.g. mackerel, tuna, bonito, sardines, marlin and butterfly kingfish), which might result in 'scombroid (histamine) poisoning'. Bacterially converted histidine to histamine might lead to severe reactions and even death and once the fish is contaminated with this toxin freezing or cooking will not be effective in removing the toxin. Paralytic shellfish poisoning (PSP) and saxitoxin (STX) poisoning as a result of dinoflagellate algae contamination of the shellfish can also be another risk to be aware of for travellers.

In this joint issue our articles will cover some of the diseases of travel such as syphilis, avian influenza, dengue and mosquito-transmitted viruses, Zika as well as antibiotic resistant bacterial infections and food borne diseases involving human beings. Related to plants and animals articles will cover Chytridiomycosis, blue tongue, decline in bees, crop diseases. Disease surveillance and biosecurity aspects are also included. Australia and the UK have historic links and extensive travel history and we are overjoyed to put a joint issue together and thank all the contributors, Editorial Boards of the both journals and Editor-in-Chief of *Microbiology Australia* Ian Macreadie and the Digital Communications Manager, Microbiology Society, UK, Ruth Paget for their support during the production.

Reference websites referred to in the above article are:

- http://www.who.int/ith/diseases/en/
- https://wwwnc.cdc.gov/travel/diseases/
- http://www.who.int/tb/publications/2006/who_htm_tb_2006_363.pdf

Biographies

Dr Kurtböke has been working in the field of biodiscovery and has been a member of the international actinomycete research community since 1982. She currently conducts research and teaches in the field of applied microbiology and biotechnology and is senior lecturer at the University of the Sunshine Coast (USC), Queensland. She has also been an active member of the World Federation of Culture Collections (WFCC) including serving as the Vice-President of the Federation (2010–2013).

Laura Bowater is a Professor of Microbiology Education and Engagement at the Norwich Medical School in the University of East Anglia with a special interest in antibiotics and antibiotic resistance. Laura is currently completing her tenure as Editor in Chief of *Microbiology Today* and this joint venture with the Australian Society for Microbiology will be her final issue in this role.

Travel and tuberculosis

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Australians frequently travel to countries with a high incidence of tuberculosis (TB). What risk does TB pose to travellers and what can be done to mitigate this risk?

In the year ending June 2016, 9.7 million Australian residents left Australia visiting one or more countries for a short term period[1]. Of the 10 most common short term destinations, six were countries with TB incidence rates of >40 per 100 000, a threshold in common usage to define 'high incidence' (Table 1)[2], contrasting sharply with the low TB incidence in Australia (5.3 per 100 000). Three of these destinations, China, Indonesia and India account for 45% of the world's total number of notified TB cases. A significant burden of multi-drug resistant (MDR) TB is noted in India and China and to a lesser extent Indonesia (Figure 1). While risks of TB to travellers have recently been reviewed comprehensively elsewhere[3] this short overview seeks to address key questions in understanding TB in the context of travel.

Table 1. Top 10 countries of destination for short term Australian travellers (in order of frequency)

Country	TB incidence per 100 000 (2014)[2]
New Zealand	7.4
Indonesia	399
USA	3.1
United Kingdom	12
Thailand	171
China	68
Singapore	49
Japan	18
Fiji	67
India	167

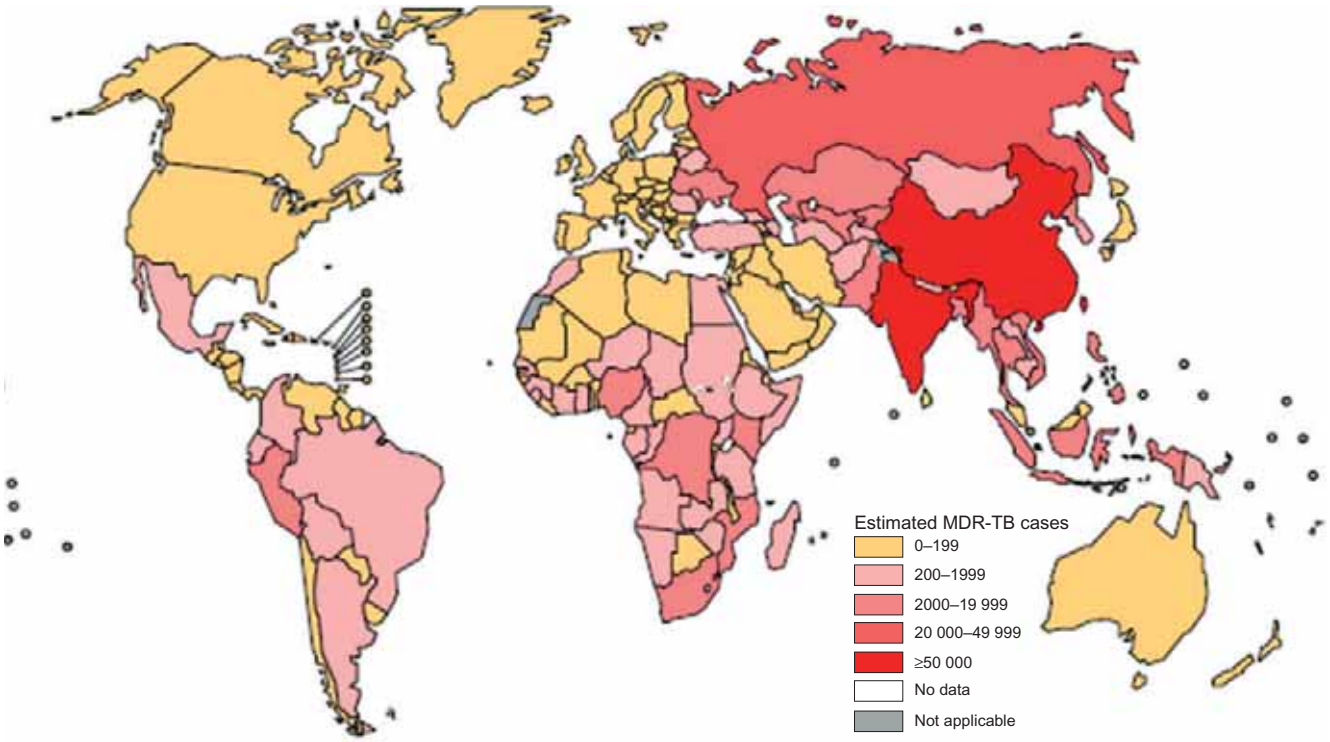

Figure 1. Number of MDR-TB cases estimated to occur amongst notified pulmonary TB cases, 2014 (reproduced from World Health Organization[2], figure 4.6, p. 64).

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Assessing the risk

The likelihood of acquiring TB is determined by the probability of encountering one or more infectious cases and the cumulative duration of exposure. Host factors increase the risk that asymptomatic infection, (latent TB (LTB)), will progress to active disease and how severe that disease may be. In addition disease is most likely to manifest in the 2–5 years following acquisition.

The risk of acquiring infection for travellers, as assessed by tests for LTB such as the tuberculin skin test (TST) and interferon gamma release assays (IGRA), broadly is proportionate to the TB incidence in country of destination and the duration of stay. However this is not always the case and risk is substantially affected by variations in TB incidence in-country, living circumstances during the period of travel and activities pursued during travel. Participation in healthcare in countries of high burden is particularly acknowledged as both a risk for contracting TB and for the TB strain to be drug resistant. 'Medical tourism', seeking elective surgery overseas, is commonly to countries of high TB burden and may present additional risks of nosocomial or community transmission of TB.

Travellers previously treated for TB remain at risk of a new infection in communities with high rates of TB transmission.

Some of the most devastating manifestations of TB such as miliary TB and meningitis are more likely to occur in children under the age of five years and especially those less than age 2. Young children accompanying their migrant parents to countries of high TB burden to visit relatives and friends may be exposed to a significant risk of TB, especially if a household contact has untreated tuberculosis, regardless of the risk of TB as determined by overall country incidence.

Getting there and getting about

While the confined space of an aircraft may suggest an ideal environment for the spread of TB by respiratory aerosols, this is not the case. There are no published cases of active TB which have been demonstrated to have been acquired during plane travel. A recent systematic review provides evidence that infection may be transmitted on aeroplanes but 14 of 21 publications assessed did not find any evidence of transmission even when the index case was AFB smear positive[4]. Only one publication provided substantial evidence that TB infection (without disease) had been transmitted during air travel[5]. Modern passenger aircraft have sophisticated air flow management with HEPA filtered air efficiently being removed from the cabin in a downward direction (Figure 2)[6]. International convention considers only those passengers in the same row as an index case and 2 rows in front and two rows behind to be potentially at risk[4]. Contact tracing is not generally embarked upon unless the flight duration is 8 hours or longer. Compared to most countries, relatively few international flights to and from Australia and New Zealand are of shorter duration.

Figure 2. Cabin airflow (reproduced from European Centre for Disease Prevention and Control[6]).

While TB transmission in public ground transport may well occur, duration of exposure is short and the risk is difficult to quantify as passenger identity and detailed records of passenger seating is not usually recorded[7]. Crowded waiting rooms, especially if poorly ventilated could be a particular risk.

Prevention

TB prevention strategies should focus foremost on those with the greatest risk of mortality or long term disability. As such, prevention of TB in travelling children should have the highest priority. Although no longer used routinely in Australia, BCG vaccination in early childhood reduces the risk of disseminated disease and TB meningitis by >70%. The *Australian Immunisation Handbook*[8] recommends BCG vaccination for children, especially under the age of 5 who will be travelling and staying in countries with an annual TB incidence of 40 or greater per 100 000 population for a prolonged period. At the current time such an effective preventative strategy is not easily implemented as there is no registered BCG product available in Australia (as of 1 January 2016) and there is variable usage between State and Territory jurisdictions of 'BCG 10', a vaccine manufactured in Poland and not registered in Australia by the Therapeutics Goods Administration. This vaccine shortage is in the context of a global shortfall in BCG vaccine supply[9], a situation which is unsatisfactory and remains unresolved.

In the absence of BCG vaccine, post travel testing for LTB is an alternative and also applicable to older children and adults where BCG is generally not recommended. Two to three months

following return from a prolonged stay in one or more high TB burden countries, the TST or IGRA can be performed to assess whether LTB is present. While a pre-travel test can strengthen the conclusion that exposure has been recent, this is not necessarily required in young children from low TB burden settings such as Australia. If there is evidence of LTB, then treatment ('chemoprophylaxis') should be offered. For children, a three months course of isoniazid and rifampicin is well tolerated and effective. Isoniazid for 6–9 months is the most commonly used regimen in adults. The use of rifapentine-containing regimens is difficult as the agent is not registered in Australia.

Special situations

Once acquired, the risk of TB disease in HIV infected persons is generally estimated as 10% per annum as opposed to 5–10% lifetime risk for immunocompetent persons. While this risk is mitigated by immune maintenance or restoration by highly active antiretroviral therapy, HIV infected travellers should be counselled about their risk. Suggestions to use isoniazid as a pre-exposure prophylaxis for short-term travellers[10] are not supported by evidence and may unnecessarily cause harm by hepatotoxicity, particularly in older subjects.

The risk of progression from latent to active TB is increased in pregnancy and is associated with a risk of congenital TB, increased risk of foetal loss as well as harm to maternal well-being[11]. While drug susceptible TB can be treated in pregnancy with standard therapy, some of the drugs used to treat MDR-TB are considered to be potentially teratogenic and pregnant women should consider changing their travel plans if their destination involves a prolonged stay in a community where the risk of MDR TB is high. If this is not possible, avoidance of congregate settings and non-essential visits to healthcare facilities would be prudent.

Travellers working in healthcare overseas where TB risk is increased should be educated regarding personal protective equipment use and have a baseline TST. While there is no unequivocal evidence that BCG administration to adults prevents TB, BCG can be considered for TST negative healthcare workers who are likely to work in a setting of high MDR/XDR TB burden[12]. For immunocompromised people including those living with HIV and in pregnancy, BCG is contraindicated.

As IGRA tests, unlike the TST, are unaffected by prior BCG, they can be used to assess LTB in healthcare workers who are TST positive at baseline. TST negative HCWs who do not receive BCG can be monitored by TST periodically during deployment or after return. Serial IGRA testing in HCWs who are initially IGRA negative is complicated by spontaneous conversions and reversions and some authorities advise against its use in this setting[13].

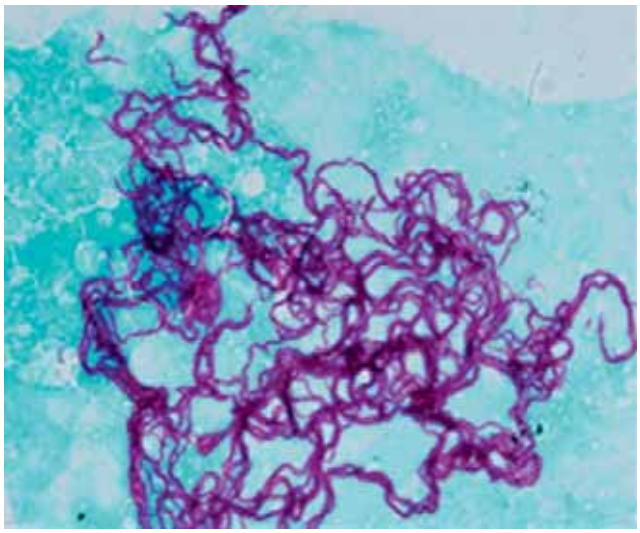

Figure 3. Typical cording of *M. tuberculosis* isolated from liquid culture (photo courtesy of Dr Sushil Pandey).

Post travel assessment

It is important that active TB is considered diagnostically at the first point of contact with the health system if a returned traveller who has visited a high TB burden country presents with TB symptoms, especially >2 weeks of cough, fevers and weight loss in adults and fevers, cough, lymphadenopathy, failure to thrive or neurological disturbance in children. Chest radiography and sputum AFB smear and culture for mycobacteria (Figure 3) and, where drug resistance is suspected, rapid molecular tests such Xpert MTB/RIF are the mainstay of the diagnostic approach. Tests of LTB should not be used to diagnose or exclude active TB as both false positive and false negative results are problematic. In contrast, evidence of recent acquisition of LTB should prompt either initiation of chemoprophylaxis or regular clinical and radiological review for at least 2 years. Australia has a well co-ordinated network for TB control and expert advice on treatment and prevention of TB can be readily obtained.

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Biography

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Avian influenza. Why the concern?

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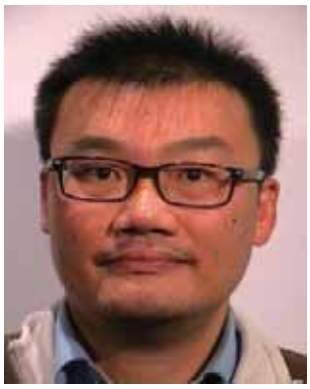

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Avian influenza normally has little impact on poultry and wild birds but since 1996, highly virulent viruses have emerged and continue to circulate in many countries. The results of these viruses have been devastating in domestic poultry and they have also spilled over into humans, infecting and killing hundreds and raising the opportunities for the virus to further adapt and possibly cause a future influenza pandemic. This article briefly details these events and discusses the consequences of these viruses continuing to circulate and cause disease.

The year 2016 marks a milestone for avian influenza, often referred to as 'bird flu'. It is now 20 years since the outbreaks of highly pathogenic avian influenza (HPAI) in farmed geese in the Guangdong Province of southern China[1]. These viruses are now recognised as the progenitors of the zoonotic H5N1 avian viruses that caused global concern in Hong Kong in 1997 with 18 human cases and 6 deaths and led to extensive poultry culling and changes to live bird markets (Figure 1). These viruses continued to evolve and rapidly spread from China throughout Asia resulting in a HPAI panzootic (a global disease epidemic in animals) event which continues to this day. The 'H' in H5N1 refers to the serotype of the haemagglutinin protein and the 'N' is the serotype of the neuraminidase protein. H and the N proteins are both abundant on the surface of the influenza virus and play key roles in the attachment (H) to cells and the release (N) from infected cells. The H protein has a proteolytic cleavage site (PCS) where host proteases cleave the protein into two subunits HA1 and HA2, an essential step in producing infectious virus. Only strong proteases present in the respiratory tract of mammals and birds, and the gastrointestinal tract of birds, cleave the H of low pathogenicity influenza viruses (LPAI). However, in HPAI the H protein acquires

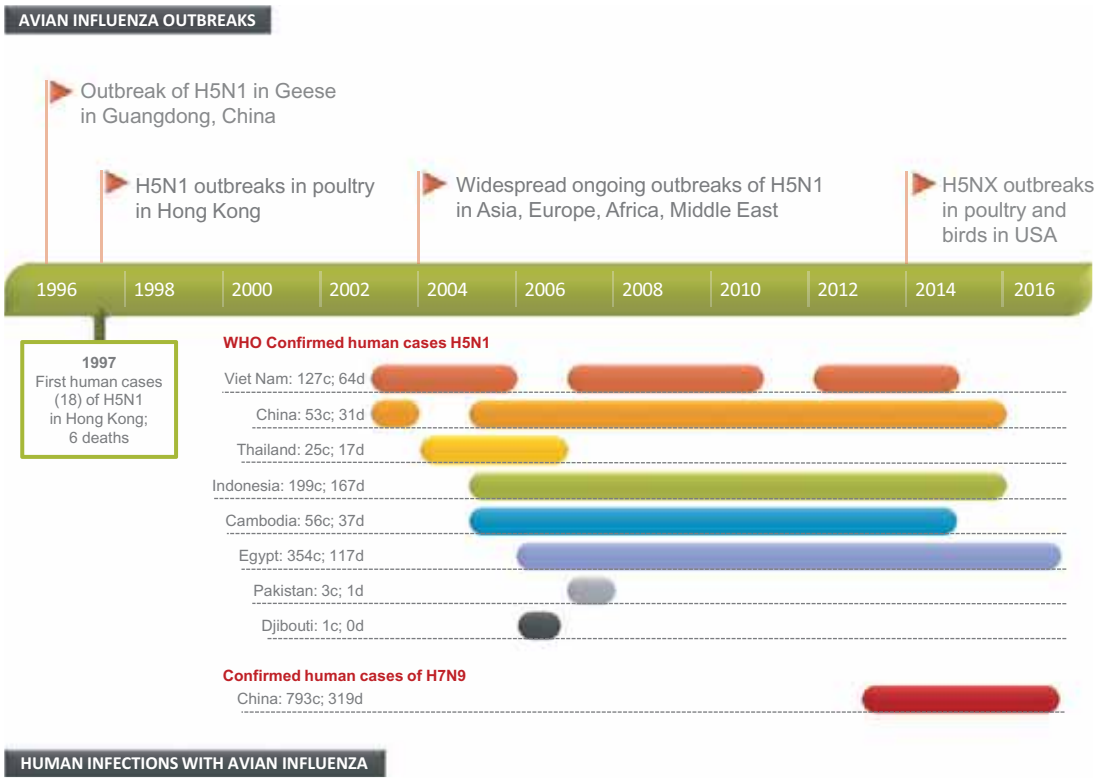

Figure 1. Timeline showing the spread of main countries affected by avian H5 viruses since 1996 and the cases of human H5/H7 infection (c = cases, d = deaths) reported to WHO.

an insert of additional basic amino acids at the PCS allowing proteases in many organs to cleave the protein and virus to spread widely in the body. These HPAI viruses can be extremely deadly in some poultry species especially chickens and can lead to high mortality in flocks within a day or two of infection. HPAI viruses with the multi-basic PCS have been restricted to the avian influenza H5 or H7 types to date. The majority of influenza viruses circulating in avian species lack this insert and therefore do not cause significant disease in birds (including most H5 and H7 viruses).

The H5N1 HPAI problem

So what's the problem 20 years on? Essentially it is the ongoing persistence of HPAI and its wide geographical spread (Figure 2) that have resulted in millions of birds being infected along with some humans, mostly through contact with infected poultry. The consequences of this panzootic have been dramatic, with millions of birds culled or dying from infection with H5N1 viruses. While human infections have been much fewer to date (854 human infections reported to WHO as of 19 July, 2016), the outcomes have been severe, with 450 deaths, fortunately without sustained human to human transmission[2]. The lost food supply and the costs and effort in vaccinating or culling birds, monitoring outbreaks and treating infections during this period have been enormous. Concomitantly has been the ongoing threat that these viruses, either by mutation, genetic reassortment or a combination of the two, might generate a virus that is more transmissible between humans and could cause a major human pandemic. Reassortment, where two or more influenza viruses infect the same cell resulting in a mixture of virus genes in the progeny can produce the most dramatic changes resulting in a unique virus. This has happened scores of times since 1996, most recently in North America and China where the H5 viruses have expanded their N type repertoire with HPAI H5N2 and H5N8 outbreaks in chickens and turkeys in North America and both avian and human infections in China with H5N6 from 2014–16, leading to these viruses being now referred to as H5Nx viruses. To date these viruses have retained their avian characteristics and have only occasionally infected humans: for example, there have been 14 cases of human infection with H5N6 reported in China since 2014.

A new problem, H7N9 LPAI

Adding to this mix of H5 viruses have been other avian influenza viruses that have also caused significant human infections. The most serious of these recently have been H7N9 LPAI infections, first detected in March 2013 in Southern China that have since recurred annually, with at least 793 human cases and 319 deaths, mostly associated with exposure to H7N9 infected birds especially with elderly men at places like live bird markets (Figure 3). As with H5 HPAI, these sporadic human H7N9 infections from birds and the continued endemic circulation in live bird markets and farms

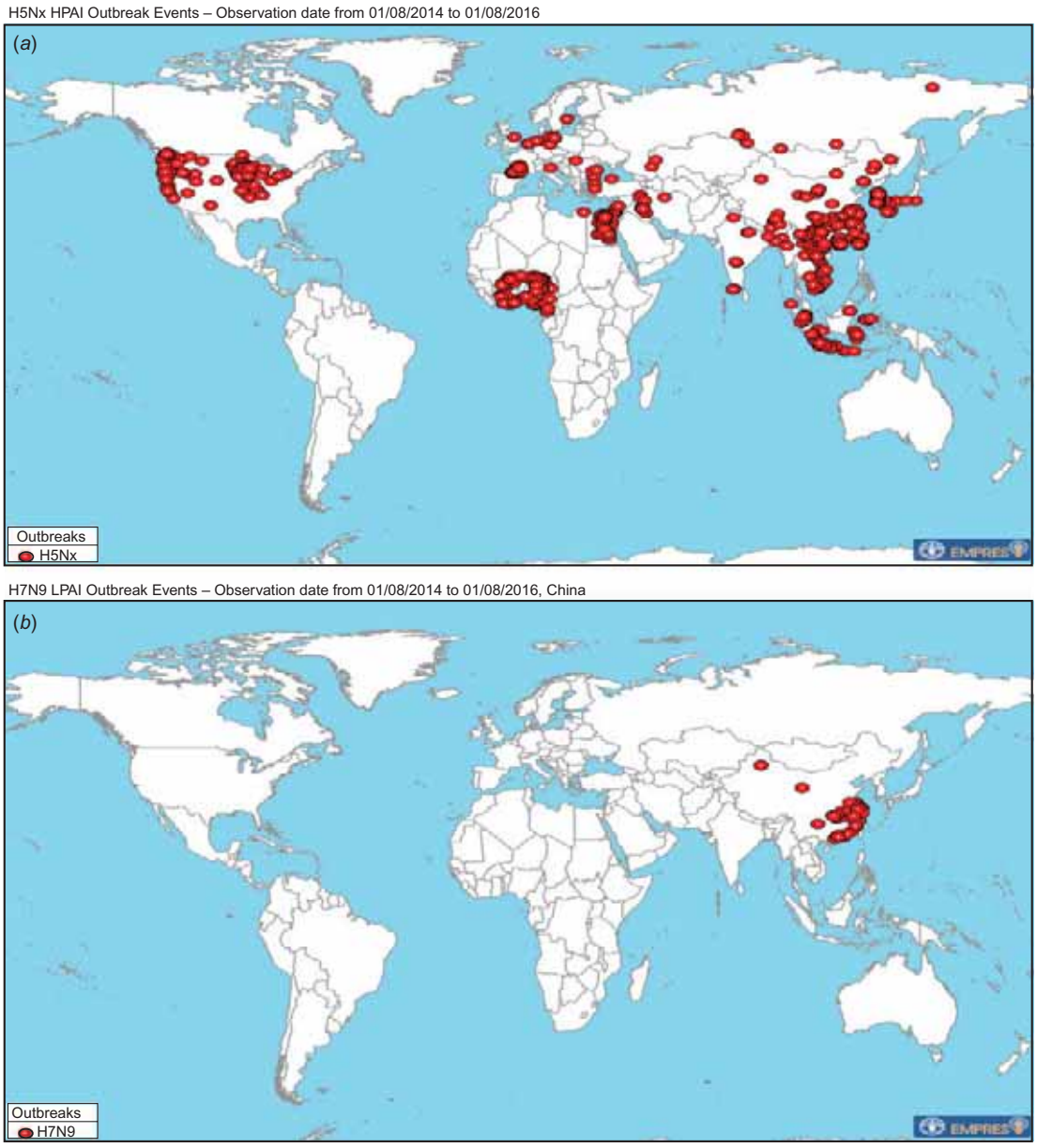

Figure 2. Red markers show outbreaks of (*a*) A(H5N1) and A(H5NX) HPAI or (*b*) A(H7N9) LPAI in birds in the last 2 years (figure produced by Ahmed Al-Naqshbandi, Animal Production and Health Division-Animal Health Service, FAO, using the EMPRES database: http://empres-i.fao.org/eipws3g/).

in China, mean that the public health risk from exposure to these and potentially novel reassortant viruses remains a great concern. Other influenza A subtypes such as H9N2, H10N8 and H6N1 have also been implicated in human infections in China, some of which have been fatal, while others such as H7N7 cases in the Netherlands, have been associated with much milder human infections. As endemic LPAI viruses such as the Chinese H7N9 and H9N2 viruses have little pathogenicity in poultry, there is little warning of their presence, resulting in increased risk of human exposures.

The big questions that still remain today

Can these viruses be eliminated or controlled in poultry and if not, will any adapt and increase their tropism for humans, leading to widespread outbreaks or a potential human pandemic of unknown severity? Thankfully the H5N1/H5Nx HPAI viruses have so far failed to become more transmissible in humans, with only a few possible clusters of H5N1 human-to-human transmission and there has been little increase in the number of human cases even with ongoing poultry outbreaks and human exposure. This is supported by testing in ferret models of influenza where H5Nx viruses did not transmit from infected to naïve animals even when co-housed, nor could they be transmitted to ferrets via virus infected aerosols. The situation for recent Chinese H7N9 viruses is less clear cut. Similar to H5Nx, there have been few human secondary infections or infection clusters recorded to date, but ferret studies demonstrated that these H7N9 viruses were easily transmitted by close contact and even by aerosols, although still not

Figure 3. A typical live bird market in Asia (photo supplied by Paul Selleck).

as efficiently as human seasonal influenza A viruses. The possible emergence of a virus variant of these or other subtypes that is able to replicate and transmit by the aerosol route more efficiently in man, would be an immediate pandemic concern since modern air travel means that infected persons can easily and quickly spread their infections at a global level before transboundary infectious disease mitigation strategies can be effectively implemented. This was highlighted by the rapid worldwide spread of the 2009 pandemic H1N1 virus that emerged from swine. The establishment of the Asian lineage H5N1 HPAI in the poultry of many other countries in Asia and Africa (Figure 2), and the recent emergence of related H5Nx viruses affecting birds in several Southeast and East Asian countries and further afield in Europe and North America, also demonstrate the potential for their widespread distribution by either cross-border poultry trade or carriage by migratory wild waterbirds.

Some possible answers?

To help manage the risk of these avian influenza viruses becoming a threat to mankind, various systems have been developed to assess avian and swine influenza viruses. One such system, IRAT (Influenza Risk Assessment Tool), was developed by the Influenza Division at CDC, Atlanta, USA. This assessment takes into account a number of factors that may be important in avian influenza viruses making the 'jump' from being an avian virus to becoming a human virus. These include both virus and host characteristics, such as virus' H receptor specificity, pathogenicity in man and in animal models, background levels of immunity in the human population, transmissibility in man and in animal models, the number of infected birds and many other factors. These factors are combined and influenza virus types are ranked by plotting them according to their potential risk to achieve 'sustained human-to-human transmission' (emergence risk) and potential 'for significant impact on public health' (impact risk). In a recent publication[3] from a small number of existing avian influenza viruses tested in the IRAT model, the Chinese H7N9 virus achieved the highest score (above H5N1 and a swine virus H3N2v) and this risk factor had increased slightly since a previous assessment in April 2013. This is not to say that an H7N9 outbreak is imminent but these rankings are meant to help guide public health measures such as early vaccine development and to also encourage virus control in the avian population, or to introduce measures to avoid human infection.

In addition to these risk assessments, researchers are exploring the factors that allow interspecies transmission but limit human transmission. A recent study with an H7N9 isolate suggests a 'genetic bottleneck' during infection of ferrets, and possibly humans, whereby the virus becomes less fit and therefore unlikely to be easily transmitted[4]. However, until we fully understand the mechanisms that allow ongoing human to human transmission it would be prudent to try to eliminate from poultry flocks those viruses with the highest risk to man (e.g. H7N9) and those of greatest risk to the domestic poultry population and to the global food supply (e.g. H5Nx HPAI) by targeted culling, effective poultry vaccines or in the future breeding poultry genetically resistant to HPAI. Meanwhile it remains important to maintain surveillance for novel influenza viruses in animals and humans and plan measures to combat any emerging virus in the human population, including appropriate vaccines and effective anti-viral drugs.

Acknowledgement

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Biographies

Professor Ian Barr is the Acting Director of the WHO Collaborating Centre for Reference and Research on Influenza based at the Doherty Institute in Melbourne. He joined the Centre 16 years ago and has previously worked in commercial, research and academic scientific positions. The Centre is one of six in the world that supports a WHO-led global human influenza surveillance network.

Dr Frank Wong is a Research Team Leader with the CSIRO Australian Animal Health Laboratory. Frank is the current World Organisation for Animal Health (OIE) expert focal point on avian influenza for Australia. He is also a contributor to the Joint OIE/FAO Network of Expertise on Animal Influenza (OFFLU), and currently represents OFFLU at the World Health Organization (WHO) vaccine consultations on zoonotic influenza viruses for pandemic preparedness purposes. He also serves as a Steering Committee member of the Wildlife Health Australia National Avian Influenza Wild Bird Surveillance Program. Altogether, Frank has more than 15 years' experience in the molecular characterisation of microbiological pathogens.

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Dengue and the introduction of mosquito-transmitted viruses into Australia

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Dengue virus outbreaks involving 100s of cases periodically occur in north Queensland, the area of Australia where the primary mosquito vector, *Aedes aegypti*, occurs. This article summarises the ecology, history, current situation and control of dengue virus transmission in Australia and examines the threat posed by newly emergent arboviruses, such as Zika and chikungunya viruses.

Dengue viruses

Each year, across tropical and sub-tropical regions of the world, an estimated 390 million people are infected with one of the four serotypes of dengue virus (DENV). The DENVs are single-strand positive sense RNA viruses of the genus *Flavivirus*, which also includes mosquito-borne viruses such as yellow fever (YFV), Zika (ZIKV), Japanese encephalitis (JEV) and West Nile (WNV) viruses. About a quarter of infections with DENV are symptomatic. So called 'classic dengue fever' is characterised by fever, rash, headache, and muscle and joint pain. Severe and potentially fatal disease occurs in about 1% of cases of DENV infection. This is characterised by plasma leakage with or without haemorrhage.

The DENVs are predominately transmitted between humans by the mosquito, *Aedes aegypti*, which has adapted its lifestyle to human habitation. Biological traits of this species that enhance its ability to serve as a vector of DENVs are: (1) it feeds almost exclusively on humans; (2) multiple blood feeding behaviour whereby a single female can bite several times to obtain a bloodmeal, thus potentially infecting numerous people; (3) adaptation to use man-made containers, such as tyres and potplant bases, as larval habitats; and (4) it prefers to feed and rest indoors.

The Asian tiger mosquito, *Aedes albopictus*, can also efficiently transmit DENVs and has driven outbreaks in locations where *Ae. aegypti* is absent or are in low numbers. However, *Ae. albopictus* does not share the same anthropophilic ecology as *Ae. aegypti* and so outbreaks caused by this species are generally not of the same magnitude as those driven by *Ae. aegypti*.

Dengue in Australia

The DENVs are not endemic to Australia, but are intermittently introduced by infected travellers. Disease attributed to DENV infection has occurred historically in Australia since the 1800s. Early outbreaks involved 1000s of cases and whilst primarily focussed in northern Queensland, transmission extended as far south as Gosford in New South Wales. For instance, the 1904–05 epidemic in Brisbane infected 75% of the population and was associated with 94 deaths. One of the largest and most widespread epidemics occurred in 1954–55 and it was estimated that at least 15 000 people were affected in Townsville alone. Following this epidemic, outbreaks of dengue ceased in Australia for 26 years, coinciding with a contraction in the geographical range of *Ae. aegypti*. This contraction was due to a decline in rainwater tank usage via reticulation of water supplies, improved sanitation, use of residual insecticides by homeowners, and the invention of the motor mower and the resulting improvement in the maintenance of domestic backyards.

DENV reappeared in 1981, causing an outbreak across multiple localities in north Queensland. The frequency of dengue outbreaks has increased dramatically in the last 25 years. This increase can be attributed to a number of factors, including (1) epidemic DENV transmission in neighbouring countries; (2) increased arrivals from dengue active countries into international airports in Cairns and Townsville, which were opened in the mid-1980s; and (3) high populations of *Ae. aegypti*. Tourists taking advantage of low cost flights to Bali, fly-in, fly-out workers to Papua New Guinea and family visits have accounted for a considerable proportion of imports into Cairns in recent years. Although there have been almost 50 outbreaks in this time, they are usually of short duration and involve less than 100 cases. Larger outbreaks sporadically occur, involving 100s of cases, often across multiple locations and over several months (Figure 1). The largest outbreak in 50 years occurred in Cairns in 2008–09 when almost 1000 cases of DENV-3 were reported. The explosiveness of this outbreak was attributed to unseasonably hot weather and above average rainfall leading into the wet season, coupled with delays in case notification and

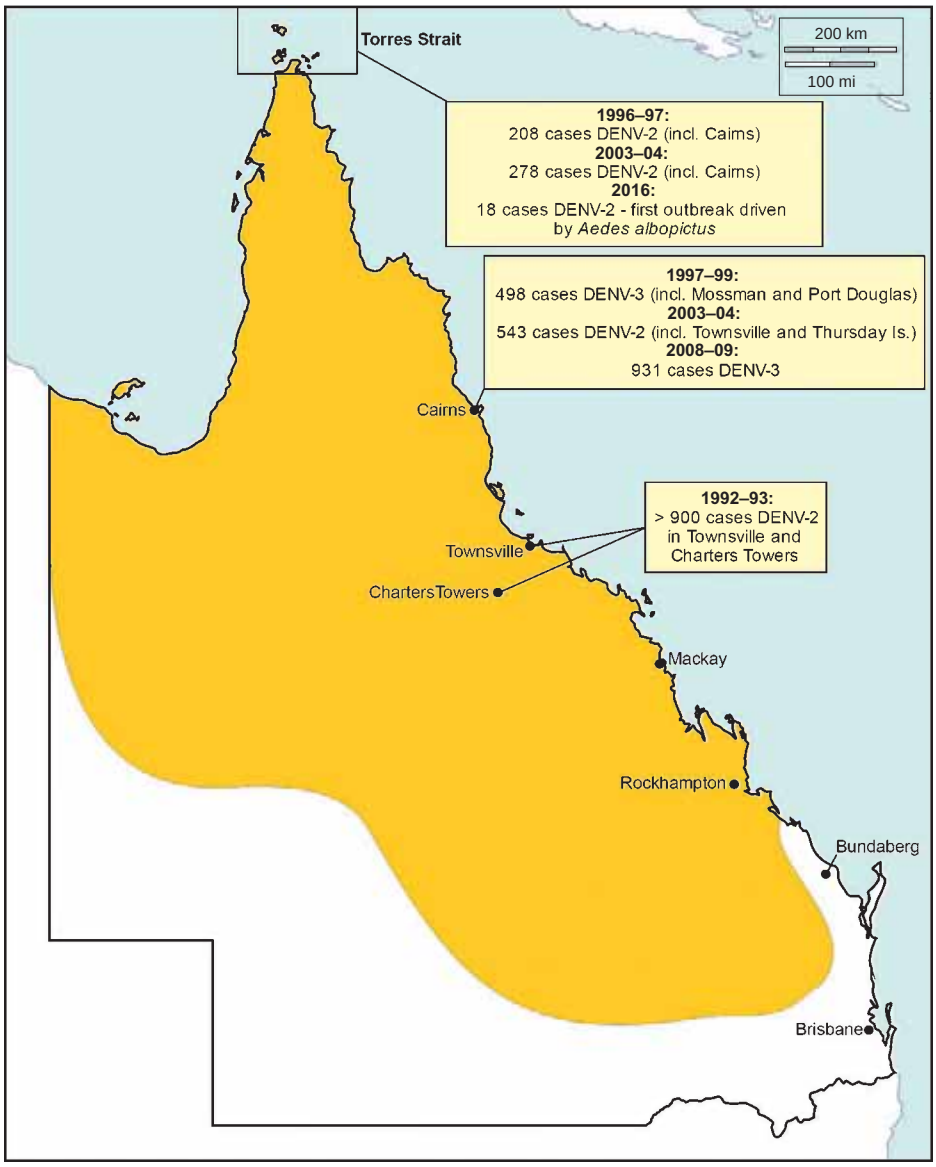

Figure 1. Map of Queensland showing the current range of *Aedes aegypti* (shaded gold) and significant dengue outbreaks since 1992. The locations in parentheses also reported cases that were linked to the primary epicentre of the outbreak. The distribution of *Aedes albopictus* is restricted to the Torres Strait.

a shortened extrinsic incubation period of the DENV-3 strain in *Ae. aegypti*.

Dengue control in Australia

In the absence of an effective DENV vaccine or specific antiviral treatment, the primary disease control strategy is suppression of *Ae. aegypti* and/or *Ae. albopictus* populations. This involves elimination of containers in which larvae develop by removing them or treating them with methoprene, an insect growth regulator which interferes with mosquito metamorphosis. Adult control is also implemented during episodes of local transmission and involves targeted spraying of indoor resting places of *Ae. aegypti* with residual pyrethroid insecticides. Considerable success has been achieved in the control of *Ae. albopictus* is the Torres Strait when harbourage spraying of resting sites with pyrethroids has been used to supplement larval control. The success of interventions to limit local transmission is dependent on timely notification of suspected cases. Delays in case notification can result in a second generation of cases before control measures are initiated, which contributes to the rapid acceleration of some dengue outbreaks.

Although they are effective, chemical-based control methods are relatively expensive, labour-intensive and there is the potential for mosquitoes to develop resistance to the insecticide being applied. A promising control strategy that had its first field evaluations in north Queensland in 2011 is the release of *Ae. aegypti* trans-infected with the endosymbiotic bacterium *Wolbachia*. *Wolbachia* reduces the ability of the mosquito to transmit DENVs. Field releases in the Cairns region and Townsville have been very successful,

reaching almost 100% fixation of *Wolbachia* within the *Ae. aegypti* population. Encouragingly, strong DENV blocking within the mosquito continues at least one year post release. Because this technology is still in its infancy, it is too early to determine the effect of *Wolbachia* releases on the frequency and magnitude of DENV transmission amongst the human population in north Queensland.

The global march of other *Aedes*-transmitted viruses

Although local transmission of DENVs occurs all too regularly, Australia has so far been spared from the spectre of chikungunya virus (CHIKV) and ZIKV, which are also transmitted by *Ae. aegypti* and *Ae. albopictus*. CHIKV causes crippling arthralgia and has undergone a global expansion since 2004 that has afflicted millions of people on multiple continents. ZIKV has gone from causing an obscure non-specific febrile illness to being associated with neurological disease syndromes including Guillain-Barré syndrome (a form of paralysis) and congenital birth defects, most notably microcephaly, during its march through the western Pacific and South America. Despite over 550 and 66 imported cases of CHIKV and ZIKV, respectively, being notified in Australia as of August 2016, local transmission has not been reported. This is likely because most cases have been reported outside of areas where *Ae. aegypti* and *Ae. albopictus* exist and comprehensive control actions have been rapidly undertaken in response to notified cases in north Queensland.

Restricted to Africa and South America, YFV causes episodic outbreaks of acute haemorrhagic disease which have the potential to spread to other areas of the world, as evidenced by 11 imported cases into China from an outbreak in Angola that began in December 2015. However, the risk of a YFV epidemic occurring in Australia is expected to be low, as travellers from endemic areas must be vaccinated against the virus.

The future

Due to the presence of *Ae. aegypti* and infected travellers, the DENVs will continue to be a threat to north Queensland. Current control programmes have undoubtedly limited the severity of DENV outbreaks and the *Wolbachia*-based approach may provide an alternative to the use of insecticides in the future.

Any geographical expansion of *Ae. aegypti* or the establishment of *Ae. albopictus* in temperate regions could render populated cities of eastern Australia, such as Brisbane and Sydney, receptive to outbreaks of DENVs, CHIKV or ZIKV. Comprehensive surveillance to detect the presence of these two species and rapid response protocols are essential to prevent their establishment.

Biography

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Pregnancy, the placenta and Zika virus (ZIKV) infection

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Zika virus (ZIKV) infections have been recognised in Africa and Asia since 1940. The virus is in the family *Flaviviridae* and genus *Flavivirus*, along with Dengue, Japanese encephalitis virus, Tick borne encephalitis, West Nile virus, and Yellow fever virus. These viruses share biological characteristics of an envelope, icosahedral nucleocapsid, and a non-segmented, positive sense, single-strand RNA genome of ~10 kb encoding three structural proteins (capsid C premembrane/membrane PrM/M, envelope E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). ZIKV has three known genotypes; the West African (Nigerian cluster), East African (MR766 prototype cluster), and Asian strains. Virus sequencing from the most recent South American outbreak suggests this virus is related to the 2013 French Polynesian isolates of Asian lineage.

ZIKV like other flaviviruses is arthropod-borne (arbovirus), with more recent evidence for sexual transmission, persistent presence in semen[1], and higher rates of acquisition due to a higher reproductive number than Dengue virus (DENV)[2]. Infection with ZIKV is usually asymptomatic (~80% of cases) or causes mild disease similar to less severe DENV. However, ZIKV has emerged as a major public health threat globally due largely to substantial recent outbreaks in areas of large gatherings, and observed association with fetal neurological damage including microcephaly in the Americas and elsewhere (reviewed in Marrs *et al.*[1]). Countries involved in the most recent outbreaks are summarised in the associated paper here. As a result of the risk to pregnant women, Australian public health authorities (and those in many other countries) recommend pregnant women defer travel to high risk countries. However, if exposure is likely, these women should prevent mosquito bites, have their sexual partners avoid mosquito bites, and post exposure avoid pregnancy for 8 weeks (summarised in Marrs *et al.*[1]), or possibly longer.

Clinical outcomes of mother to child transmission and diagnostic difficulties

Mother to child transmission (MTCT) of ZIKV has been documented via placental infection and damage, with increasing evidence of fetal ZIKV microcephaly. The number of infected mothers, compared with number of infected fetuses (i.e. rate of MTCT), is unclear, although in one Brazilian study, of 88 pregnant women with rash before 38 weeks gestation, 82% had ZIKV and 12/42 (27%) had fetal abnormalities on ultrasound compared with 0/16 women without ZIKV[3]. However, this is likely a significant overestimate due to the method of collection, the nature of the clinic and the lack of confirmed transmission on amniocentesis. A case control study of association between ZIKV and microcephaly showed ZIKV present in mothers of 24/32 cases of microcephaly compared with 39/61 mothers of controls ($p = 0.12$), and that in the babies, 13/32 with microcephaly compared with 0/16 of the controls had ZIKV infection[4]. These rates compare with rates of MTCT in maternal cytomegalovirus (CMV) infection of 32% in primary infection and 1.4% during reactivation[5], and for rubella of 80% to 25%, depending upon gestation[6]. Effects on the fetus for all these infections depend upon many factors, including maternal immunity, gestation of infection, and viral characteristics.

Identification of mothers infected with ZIKV is predominantly via symptoms, serology, and molecular testing of the acutely infected person. Diagnosis is confounded by the low rate of symptoms (in ~20% of adults), technical difficulties with serology cross-reactivity, and the brief period of viraemia in some infections. Serology diagnostic problems occur due to co-circulation of other flaviviruses (particularly Dengue virus) in ZIKV affected areas. Cross-reactivity between ZIKV and Dengue virus occurs[7], falsely negative tests for ZIKV may result if high levels of antibody are present to other flaviruses (such as occurs following vaccination for Yellow fever virus), and acute ZIKV infection may result in false positive Dengue NS1 antigen tests[8], further confusing diagnosis. Molecular testing using nucleic acid tests such as PCR is definitive if positive, although the duration of viraemia makes identification difficult when combined with low rates of symptomatic infection.

A major concern is whether MTCT occurs in ZIKV-infected asymptomatic women resulting in unexpected fetal damage. This occurs in murine models where ZIKV tropism for cells at the maternal-fetal interface is the likely source of transplacental transmission[9], and is consistent with human cell studies *in vitro*[10]. Prolonged maternal viraemia, and excretion of ZIKV in urine for 5–6 weeks following infection provides opportunities for improved diagnosis[11], but also the possibility of continuing risk of ZIKV transmission either to other adults or MTCT during asymptomatic phases of an infected mother[1]. ZIKV has been found in breast milk in three case reports of mothers infected <3 days from delivery[12], although MTCT transmission via breast milk has not been documented.

Placental and fetal infection

Most microcephaly is thought to arise from first trimester (T1) infection, although sampling difficulties occur with the high rate of asymptomatic infection. ZIKV has been detected in fetal brain tissue from microcephalic infants, in amniotic fluid taken from mothers of affected infants[13], and from central nervous system tissue of affected microcephalic infants[14]. These are mainly observational data with minimal controls, albeit with autopsy and ultrasound data being consistent with microcephaly resulting from ZIKV infection during pregnancy[15]. ZIKV has been known to be neurotropic in animals for 60 years, with more recent murine experiments demonstrating replication in embryonic brain targeting neural progenitor cells, with consequent cell cycle arrest, apoptosis and inhibited neural progenitor cell differentiation[9,16]. This is presumed to result in the microcephalic phenotype via neuronal cell death[16]. This is consistent with observations that African ZIKV strains infect neural precursor cells in murine models (summarised in Klase *et al.*[17]).

Mother to child transmission (MTCT) studies often use models from T2 or T3 placentae, which differ from T1 placentae in structure, cell components and surface markers. Studies of infection of explanted placentae in other viral infections such as with CMV show neonatal neural malformation and intra uterine death may be caused partly through Th1 cytokine-induced placental damage[18,19]. The placenta is a complex organ that changes significantly over pregnancy, and comprises some unique cells with differential susceptibility to viral infection (Figure 1). ZIKV infects isolated placental primary cells and human placentae cultured *ex vivo*, with mid pregnancy (T2) chorionic villi (cytotrophoblasts, endothelial cells, fibroblasts, Hofbauer cells) and amniochorionic membranes (amnion epithelial cells, trophoblast progenitors) infected[10]. As MTCT requires virus to traverse the placenta, the role of trophoblasts (either as differentiated syncytiotrophoblasts or cytotrophoblasts) is likely to be key, similar to the key role they have for MTCT

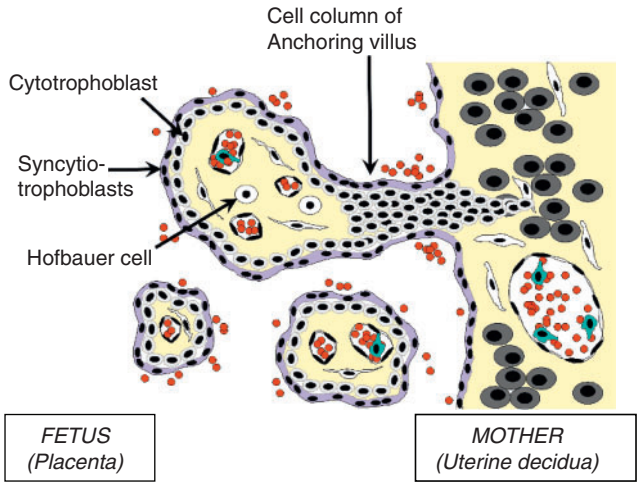

Figure 1. Possible sites of Zika virus infection of the human placenta. Cytotrophoblasts (CTB) form the inner layer of villi, fuse into multinucleated syncytiotrophoblasts, or give rise to extravillous trophoblasts (EVT), which invade and migrate into maternal uterine decidua (that is from left to right in the figure). Viruses infect different cell types, with ZIKV shown in *ex vivo* explants to infect CTB, endothelial cells, fibroblasts, and Hofbauer macrophage-like cells in the villus core on the uterine decidual side (Tabata *et al.*[10]).

of other viruses[18]. Placental inflammatory response to ZIKV may be important in fetal neurological pathology, although this remains to be proven in humans.

Early gestation (T1) infection with ZIKV has been associated with miscarriage, intrauterine growth restriction, and microcephaly[14], and although causation is likely, it is still to be proven. These changes result from direct infection of the fetal neuronal tissue, although placental infection may contribute to the more generalised fetal pathology as occurs with other viruses causing similar fetal pathology, possibly through virus-induced cell cycle dysregulation[20]. The presence of receptors and cell entry cofactors on these cells (Axl, Tyro3, TIM1) which are known also to be bound by other flaviviruses (DENV – Tyro3, Axl, Mertk) suggest a common mechanism of entry may exist[21]. These receptor tyrosine kinases are from a family known to clear apoptosed cells and interact with the innate immune system. Interventions that prevent ZIKV binding to these may provide therapeutics that can be trialled in mouse models or human placental explant models where reduced placental damage may reduce fetal injury[9].

Future studies

ZIKV infection remains a disease clinically of either no symptoms, or relatively mild presentation with fever, myalgia, eye pain, and/or fatigue associated with a maculopapular rash. The major complication of fetal injury, particularly microcephaly and death *in utero*, need to be addressed with further research. Good murine and human placental explant models exist[18], and candidate targets for ZIKV cell binding inhibition have been identified[10]. Vaccines for

related flaviviruses are now licensed in some countries (DENV – the live recombinant Denvaxia from Sanofi Pasteur) or undergoing trials. If continued spread of ZIKV occurs either within currently infected countries, or to other naïve populations, enhanced vaccine development needs to be considered, as suggested by some commentators. If so, such a vaccine will need to prevent MTCT and address the issue of cytokine/immune-dependent injury to the fetus, transplacental transmission of ZIKV[10,18], with the potential to significantly reduce the risk of congenital ZIKV abnormalities, as has occurred with the successful use of vaccines for rubella virus. Finally, all sources of ZIKV transmission to pregnant women should be avoided, including via blood products, as these may be infected despite being from asymptomatic individuals[22].

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Biography

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Read more about Zika in the article **The voyages of Zika virus** by Derek Gatherer on page 206.

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From zero to zero in 100 years: gonococcal antimicrobial resistance






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The threat of antimicrobial resistance (AMR) in bacteria has been escalated to a rightful seat on the global health agenda. In September 2016, for only the fourth time in United Nations (UN) history, the UN General Assembly in New York will meet to focus on a health threat – antimicrobial resistance. Other diseases afforded this level of consultation at the UN were human immunodeficiency virus (HIV), non-communicable diseases and Ebola virus. There are grim predictions for the future in terms of AMR and health security that span income settings. These predictions challenge the premise that minor bacterial infections of childhood are innocuous, and threaten to halt the medical advancements dependant on antibiotic therapy. Those with compromised immune systems, whether endogenous or induced, will be at highest risk. The development and spread of AMR has been, and will continue to be, fanned by

the relentless selection pressure of exposure to antibiotics whether used appropriately, unnecessarily or suboptimally, in human health, animal health and agriculture. The distribution of antibiotic resistant bacteria is facilitated by travel and transport. Antimicrobial resistance will affect those in the community and the hospital.

A well-documented example that demonstrates the development and spread of AMR involves *Neisseria gonorrhoeae* (Figure 1). The expansion of AMR to each successive therapeutic recommendation has left limited options for treatment of this once easy-to-treat infection. International travel remains a major factor in the dissemination of drug-resistant *Neisseria gonorrhoeae* strains and this has been highlighted most recently by the global spread of strains with resistance to extended spectrum cephalosporin antibiotics, the so-called last single dose therapy. Now a combination of two antibiotics are generally recommended for the treatment of gonorrhoea, ceftriaxone and azithromycin, and resistance to both has been documented[1,2]. Resistance to azithromycin is typically caused by alteration of the 23S ribosomal RNA gene (the drug target), but may also arise via mutations causing increased activity of efflux pumps (which pump drugs out of the cell)[3]. Resistance to cephalosporin antibiotics is characterised by a mosaic penicillin binding protein-2 (PBP-2) (this mosaic PBP-2 occurs as a result of integration of DNA sequences from other bacteria producing a changed drug target). Mosaic PBP-2 strains belonging to multi-locus sequence type 1901, first reported in Japan at the turn of the millennium, have become a successful clone in several continents. Whilst genetic data are lacking to confirm what happened with the previous first-line antibiotic classes, it is likely that a similar global transmission event was responsible for limiting the use of fluoroquinolones in the early 1990s.

Many questions remain regarding how to best deal with AMR in *Neisseria gonorrhoeae*. A gonococcal vaccine remains elusive and other primary prevention strategies, such as *safer sex* behaviour change strategies have not prevented the spread of gonococcal AMR. Many regions of the world remain unaware (particularly at the population level) of the nature and extent of gonorrhoea prevalence and the incidence of antimicrobial resistance. In addition, international travel continues to threaten AMR containment and border screening is not a realistic option for preventing spread of AMR. For these reasons, ongoing monitoring of AMR, both at national and global levels, remains the central tenet of the public health response to the threat of untreatable gonorrhoea. In our opinion, this can only be achieved through combined use of both bacterial culture and molecular AMR testing strategies. Culture based surveillance remains optimal for detecting new resistance mechanisms. However, mechanism and strain-specific molecular assays add rapid, important and clinically relevant data for situational analysis and to inform treatment guidelines, to monitor the effect of interventions and to provide data in countries or remote areas with limited laboratory capacity.

Gonococcal diagnostic and AMR testing strategies in remote and regional communities of Australia provide an ideal fine scale example of the above. These communities represent one of the few globally where penicillin can still be used for treatment of locally acquired gonorrhoea. Penicillin is an ideal treatment option as it is orally administered, and can be stored without need for refrigeration hence there is considerable motivation to maintain

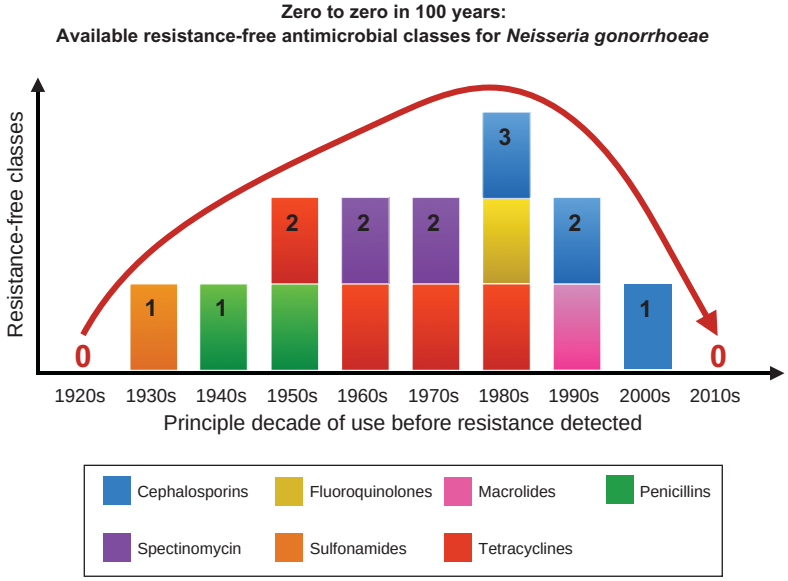

Figure 1. Gonococcal antimicrobial resistance over the past 100 years. Bars indicate the number of clinically tested antimicrobial classes available for *Neisseria gonorrhoeae* treatment.

this therapeutic option. Incursion of penicillin resistant strains from elsewhere in Australia, where resistance rates exceed 40%, or from international travellers, is an ever-present threat. Optimal surveillance is pivotal to identify such incursions and initiate rapid public health interventions. Whilst gonococcal culture is promoted, the majority of infections (~90%) are diagnosed by molecular based testing. To facilitate surveillance locally, an in-house polymerase chain reaction (PCR) assay has been developed and implemented to screen for penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains. This approach has a key benefit: rather than simply responding to emerging trends in aggregate data, the assay provides immediate results at an individual level that can readily be acted upon.

The recent incursion of the ceftriaxone resistant A8806 strain into Australia exemplifies how the dual culture-molecular approach supports public health initiatives to contain gonococcal AMR[4]. The A8806 isolate was first identified by culture (highlighting the importance of maintaining bacterial culture). After the phenotypic AMR profile was established using conventional culture-based techniques, the isolate was sequenced, and a strain-specific PCR method developed. The PCR was utilised in clinical practice to determine the spread and prevalence of the A8806 strain across the two states where the infected patient had travelled. We are currently investigating an outbreak of azithromycin resistance in Australia, and further intend to use molecular assays to gauge the extent of the outbreak. Increasingly the availability of genome sequencing is facilitating the identification and characterisation of such clusters, permitting tracking and tracing of AMR strains and investigation of transmission dynamics.

The WHO's *Report on global sexually transmitted infection surveillance 2015* shows that in many regions where disease rates are high there is limited data to determine the scope and extent of AMR[5]. This is a function of a number of factors including limited resources and syndromic management of patients. Paradoxically, best resourced settings often test relatively few gonococcal isolates for AMR, due to a preference for nucleic acid tests which do not characterise AMR profiles of well documented resistance genes. Gonococcal antimicrobial susceptibility testing remains expensive and technically difficult. Strategies are required to strengthen local laboratory capacity and capability, to increase the number of isolates for testing, with all options available to gather timely and reliable information considered.

A population-based approach that identifies those at risk for gonococcal infections, possibly linked to other health interventions, such as HIV screening of high risk people, may be reasonable. Containing gonococcal AMR should be program oriented, linking patient and contact management with the best treatments to prevent disease and reduce transmission. Merging cutting edge molecular technologies that can diagnose known and emerging AMR determinants with new ways of case finding, and bringing effective treatment to patients and partners in a timely fashion will improve health outcomes. Thus a strategy that focusses only on the acquisition of AMR data and which is isolated from other components of an active program to ensure treatment and elimination of transmission is bound to fail. Such programs may entail a shift in thinking regarding how AMR is diagnosed, how and what patients are identified, and the criteria for which treatment guidelines are modified.

Critically *N. gonorrhoeae* infects only humans and can therefore be potentially eradicated. Future success in the current context will rely on adaptive thinking, exploiting both new and pre-existing technologies to gather information and inform health care strategies. However, primary prevention must remain the principle focus.

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Biographies

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Dr Teodora E Wi, MD, FPSVI is currently the Medical Officer, Human Reproduction Team, Department of Reproductive Health and Research, World Health Organization (WHO), Geneva, Switzerland. In WHO HQ she is leading the development of STI guidelines, antimicrobial resistance in *N. gonorrhoeae* and interventions for key populations, in addition to providing technical support to regional and country offices of WHO. She has over 20 years of experience in HIV and STI programming. She was the acting team leader for HIV/AIDS and STI, Western Pacific Region Office, WHO. Prior to WHO, she was the Director, STI Capacity Raising, Family Health International (FHI) India under the Avahan India AIDS Initiative of the Bill & Melinda Gates Foundation (BMGF).

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Foodborne disease associated with travel

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The most important determinant of developing foodborne disease is travel destination. The risk is proportional to regions where there is a high level of unsanitary water supply, lack of food hygiene, lack of food safety regulation, fluctuating electricity supply and lack of education. In medium to high risk regions a travel kit, designed to prevent, minimise or treat the effects should be carried.

After a decade of comprehensive work gathering data to estimate the world burden of foodborne disease the World Health Organization (WHO) has produced a report in which it has calculated that 600 million people develop foodborne disease after eating contaminated food each year. The report also determined which regions and countries had the highest incidence and which foodborne pathogens caused the majority of outbreaks[1]. This has great significance on the varying degrees of danger of developing a foodborne disease when travelling in these regions because the most important determinant of risk is travel destination. Risk also depends on the season of travel[2].

Diarrhoeal disease represents more than 50% of global foodborne disease[1]. Traveller's diarrhoea (TD) is defined as the passage of three or more loose or unformed bowel actions accompanied by at least one of nausea, vomiting, and abdominal cramps, and may be further complicated by fever or blood in stools[2]. Depending on the destination and season of travel, the chance of developing TD when travelling can range from 30% to 70%[2].

According to the US Centers for Disease Control and Prevention (CDC) there are generally three grades of risk of developing TD[2]. The general division can be seen in Table 1.

The GeoSentinel network, the global surveillance network established in 1995 between the International Society of Travel Medicine and the CDC, has categorised the risk of travellers developing microbial gastrointestinal infections into very high, high, medium, moderate and low risk regions and has analysed the specific microbial pathogens causing gastrointestinal infections in each region[3]. Travellers to regions with high and very high morbidity due to foodborne disease were 200 and 800 times more likely to develop a gastrointestinal infection, respectively, than travellers to low or moderate morbidity such as Northern America and Europe[3].

The number of people travelling from developed to high risk regions such as Africa, the Americas and Asia increased by 60% from 2000 to 2007, and it is estimated this will continue to increase at a rate of 6% per year[3]. Currently over 100 million travellers from non-tropical regions will visit a developing country each year and 60% of travellers who visit tropical and subtropical regions will develop diarrhoea and health problems[1,3].

The risk of developing foodborne illness when travelling is proportional to regions where there is a high level of unsanitary water supply, lack of food hygiene allowing cross contamination, lack of food safety regulation when producing and storing food, fluctuating electricity supply for effective refrigeration, and lack of education and literacy[1].

Table 2 shows the general percentages of microbial pathogens that account for TD[2]. The symptoms of TD are commonly nausea, vomiting and diarrhoea but depending on the infective agent may be more severe with fever and bloody diarrhoea. The most common bacteria associated with TD are *Campylobacter jejuni, Salmonella* spp., *Shigella* spp. and enterotoxigenic *E. coli*[3]. Other pathogenic *E. coli* are also common. The main intestinal virus causing TD is Norovirus. The main protozoal foodborne pathogen causing TD is *Giardia*[2] from contaminated water used to prepare food. Other protozoa are less common. In regions where the tradition or normal practice is to eat raw or undercooked meat, poultry and eggs, drink raw milk and eat fresh produce grown using contaminated unsanitary water supplies, there is a high risk of developing the above diarrheal diseases. Infection with the tapeworm (*Taenia solium*) occurs from eating raw or undercooked pork. Some pathogens are much more common in low-income countries. These include typhoid fever and foodborne cholera. Several foodborne pathogens may cause more serious illness affecting sites outside the gastrointestinal tract including systemic, neurological, muscular, and long-term disease sequelae affecting the kidney, liver, brain, bone and skin. Travellers who are old and young, pregnant women and those with weakened immune system may be more susceptible to serious disease[1]. Some of these will be discussed further in information on specific regions, below.

The WHO African, South-East Asia and Eastern Mediterranean regions have the first, second and third highest burden of foodborne disease in the world respectively which will have major consequences for risk when travelling. The majority of cases are TD, caused by typical bacterial and protozoal agents and Norovirus. Tapeworm is also prominent, however some interesting facts have emerged from the WHO foodborne disease burden statistics in these regions that also have significance for travellers[1].

Half the global population who die of hepatitis A infection or typhoid live in the WHO South-East Asia region so both diseases must also be considered when travelling there. Hepatitis A is also prevalent in the Eastern Mediterranean region due to faecal contamination of food. This region has more than half the global cases of brucellosis and travellers could be infected from eating raw or under-pasteurised dairy products from infected cows, sheep and goats with poor domestic health regimes[1].

Table 1. Three grades of travel destination risk[2]

Risk	Region
High	Africa, Asia (not Singapore), Middle East, Mexico, Central and South America
Intermediate risk	Eastern Europe, South Africa, and some of the Caribbean islands
Low risk	United Sates, Canada, Australia, New Zealand, Japan and countries in Northern and Western Europe

Table 2. The general percentages of microbial pathogens that account for Travellers Diarrhoea[2]

Microbial foodborne pathogen	Approximate percentage of TD
Bacterial pathogens	80–90%
Protozoal pathogens	10%
Intestinal viruses	5–8%

Unlike other regions diarrhoeal disease is not the dominant foodborne disease in the WHO Western Pacific region. This area has a high incidence of liver cancer resulting from the ingestion of mouldy grain contaminated with aflatoxin. This region also has a high rate of foodborne disease due to ingestion of parasites. Most of the world's population infected with Chinese liver fluke is in this region[1]. As this parasite is contracted by eating raw or undercooked fish this should be avoided when travelling in this region.

In Central and South America, in addition to TD, toxoplasmosis and tapeworm are also important[1]. The risk of hepatitis A and foodborne amoebiasis, cysticerosis, brucellosis, infection with *Mycobacterium bovis* and listeriosis, which causes complication in pregnancy and serious systemic illness in susceptible people, have been associated with travel infections in Mexico[4].

The WHO European region has the lowest burden of foodborne disease. In first world countries non-typhoidal *Salmonella* is an issue, as it is in all regions. *Campylobacter* is also an important pathogen[1]. However, Norovirus is five times more common. One of the most frequent causes of this virus in first world countries often relates to cruise ships. Between 2012 and 2016 there were 45 gastrointestinal outbreaks on cruise ships reported to the CDC of which 41 were attributed to Norovirus[5]. Because Norovirus symptoms include vomiting, often projectile vomiting, the close quarters of cruise ships favour the rapid spread of this virus via aerosols and poor food hygiene. However, the incidence of Norovirus transmission on cruise ships is diminishing since improved sanitation and food safety and hygiene regulations and strict quarantine of infected passengers has been implemented[6].

Vaccination to hepatitis A, typhoid and cholera are available and should be considered when travelling to regions at risk of these diseases[7]. A travel kit, designed to prevent, minimise or treat the effects and symptoms of TD should be carried by travellers to medium and high risk regions. It should contain an alcohol based hand sanitiser. Often a probiotic or capsules of bovine colostrum, which can be bought over the counter, is used as a daily preventative, although studies have not proved their efficacy[2]. Anti-motility agents, such as loperimide, help reduce the frequency of bowel movements and allow travel to continue. However, the CDC does not recommended using this treatment if the general foodborne disease symptoms of TD advance to fever and bloody diarrhoea[2]. It is important for a travel kit to include dehydrated sachets of oral rehydration salts for oral hydration therapy, to reduce lost fluids and electrolytes. Oral rehydration is one of the most important treatments of TD. An antibiotic is often included in the kit, as prescribed by a medical practitioner, due to the frequency of TD's being caused by bacteria, however, travellers may find it very difficult to distinguish between the symptoms of various foodborne disease, so inadvertent use of antibiotic therapy is not recommended. Carrying a treatment for parasites such as *Giardia* is also important[2].

When travelling in high risk countries many factors, such as restaurant hygiene, are out of the traveller's control. Although the 'boil it, cook it, peel it, or forget it' rule is still highly recommended in high and medium risk regions, the hygiene of kitchens and cross contamination in food preparation areas are often unseen by travellers unsuspectingly enjoying a meal in a local or traditional restaurant. Avoiding raw or undercooked meat, fish, poultry and dairy products, exercising care when selecting food to eat, and timely use of prophylactics and medications will decrease risk and give the traveller a better chance of enjoying a trip free of foodborne pathogen health issues.

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Biography

Prue Bramwell is a Senior Lecturer in the School of Applied Science at RMIT University. She has over 20 years' experience in food microbiology and has been an educator in the fields of food microbiology and food safety for over 15 years. Her research interests are in methods for the isolation and identification of foodborne microbes.

Future issues of *Microbiology Australia*

March 2017: Bat-associated Diseases
Guest Editor: Glenn Marsh

May 2017: Industrial Microbiology
Guest Editors: Ian Macreadie and Ipek Kurtböke

Australia's biosecurity procedures and preparedness

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There is sometimes concern expressed in Australia and other countries that we do not specifically test imported food for the presence of antimicrobial resistant (AMR) bacteria. How significant is this threat and how do the biosecurity measures taken by Australia address these?

Australia operates a biosecurity regimen that is risk and science-based. Australia is one of only a few countries that has a legislated and clearly articulated Appropriate Level of Protection (ALOP) achieved through managing the risk posed by imported goods to an acceptable level. These actions include prudent sourcing, certification and testing of food products to manage the biosecurity and food safety risk.

The challenge with imported food is determining if there is a demonstrable risk. We know that AMR is a global problem, one that is estimated to threaten 10 million lives a year and a cumulative US$100 trillion of economic output by 2050 due to drug-resistant infections[1]. What we don't know is the relative contributions of factors such as inappropriate dispensing of antibiotics to the human population, resistant infections acquired through travel or hospital stays, use of antibiotics in agriculture, environmental exposure through contaminated water and soils, and consumption or preparation of foods carrying AMR bacteria.

For foods to pose an AMR risk multiple factors are likely to be in play including[2]:

- The food animals or plants are treated with or exposed to an antibiotic,
- The antibiotic is of significance to human health,
- Bacteria in or on the food animal or plant need to have resistance to that antibiotic,
- The bacteria need to survive the various stages of food processing to the extent that they are able to transmit their resistance capability to bacteria in a human host,
- The bacterium must be able to cause disease in humans, or transfer its resistance to bacteria that can cause disease in humans, and
- The disease requires treatment with antimicrobials for which the bacterium is resistant, leading to treatment failure.

In practice these criteria are rarely always met and so the contribution of food, whether imported or of domestic origin, to human AMR is not yet quantifiable and could be quite minor.

Antibiotics are used to treat and prevent disease in livestock because it is generally recognised that sick animals pose a food safety risk to humans and that livestock should be afforded good health on ethical and animal welfare grounds. Access to antibiotics for veterinary therapeutic use in Australia is controlled.

In Australia and many other countries, most food is produced without the use of antibiotics, there is some minor use in horticulture and use in livestock agriculture is largely confined to intensive rearing systems. While some livestock are provided with antimicrobials for growth promotion purposes, generally these are antimicrobials that do not impact on human health but in some countries this is not always the case. In Australia antibiotics available for growth promotion are regulated through the registration process.

Probably the most effective measure to prevent transmission of AMR through food is good food hygiene. Any good food processing and preparation process should work to reduce the number of bacteria carried forward at each step and ideally be completed with a kill step of cooking the food to remove any residual bacterial contamination. In human medicine, infection prevention and control (IPC) is an essential element contributing to any AMR strategy. Likewise, breaking the chain of transmission through good agricultural practices and good food hygiene is equally important in the food production system. If bacteria in the gut, or on the hide, of an animal can be prevented from spilling onto the meat then the risk of AMR transmission is effectively minimised. Likewise, preventing transfer of bacteria on ready-to-eat horticultural products reduces the risk of AMR transmission. Good food hygiene not only prevents exposure to AMR carrying bacteria, it also prevents food poisoning and the demand for antimicrobials.

In 2009, Australian farms produced 93% of the total volume of food consumed in Australia[3]. Over the past 20 years there has been a steady increase in the value of food imports averaging 4.8 per cent

per year[4]. Australia's food imports are generally processed, high-value products. We live in a connected world and foods from around the world are readily available in Australia. Steps are taken at airports to prevent travellers bringing in foods that pose biosecurity (and food safety) risks, but nothing can be done to address the possibly greater risk posed by the travellers themselves who may be carrying AMR bacteria from environmental exposure, contaminated food, infection or medical treatment as part of their travel.

Australia's biosecurity measures work to support our food safety objectives. We source from countries of compatible disease status and apply risk management measures (such as treatments and testing) to address potential risks. All imported food must meet biosecurity requirements before being allowed into the country and is subject to risk-based inspection at the border. To manage other biosecurity risks Australia does not import livestock and this has the side benefit of not introducing resistant bacteria through live animals into our national herd. Furthermore, chicken and pig meat imported into the country are either cooked, or further processed upon arrival, and fresh beef can only come from a few select countries.

As previously mentioned, Australia does not currently conduct AMR tests on foods at the border, just as domestic foods are not routinely tested for AMR. To conduct testing at the border in the absence of a demonstrated scientific risk and without similar testing of domestic foods would be inconsistent with our international obligations and place Australia in a vulnerable position with trading partners. Likewise, Australia would question or challenge any country that commenced testing our exported foods for AMR in the absence of a demonstrable scientific risk and an official control program.

We do not know how much of the AMR observed globally and within Australia can be attributed to food. This is acknowledged in Australia's AMR strategy, which has identified a comprehensive literature review as a key first step. The likelihood is that food's contribution is small, but it needs to be identified and possibly quantified so that measures can be devised to manage any unacceptable risk. The existing guidance[5] provided by Codex Alimentarius is valuable and is currently being reviewed.

Global interest in the increasing threat of AMR has been conveyed by consumers to the food production and retail industry with a rising number of food outlets introducing antibiotic requirements on animals sourced by their suppliers. Australia is well placed to respond to this new demand given our strong controls over critical antibiotics, our largely extensive livestock agricultural production system, and our nimble and responsive industry and government assurance systems.

As AMR is an emerging threat we do not yet have all the answers, nor all the tools. It is thought that food may contribute relatively little to this threat, but our prudent national strategy recognises the gap in our knowledge and seeks to fill this through a comprehensive literature review. Meanwhile, Australia's biosecurity system will continue to manage the risk, appropriately informed by research and experience with this rapidly developing global issue.

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Biography

Mark Schipp was appointed Australian Chief Veterinary Officer in 2011. In 2012 he was elected to the OIE Council and in 2015 was elected Vice President of the OIE General Assembly. He is chair of Wildlife Health Australia management committee and chair of Animal Health Committee. Together with the Chief Medical Officer, Dr Schipp chairs the Australian Strategic and Technical Advisory Group on Antimicrobial Resistance. Previously Dr Schipp has held positions responsible for animal derived food product inspection, market access and export certification. Dr Schipp served two terms overseas as Agriculture Counsellor in Seoul, South Korea and in Beijing, China. Mark is a biology and veterinary graduate of Murdoch University. After graduation he worked with the Western Australian Department of Agriculture.

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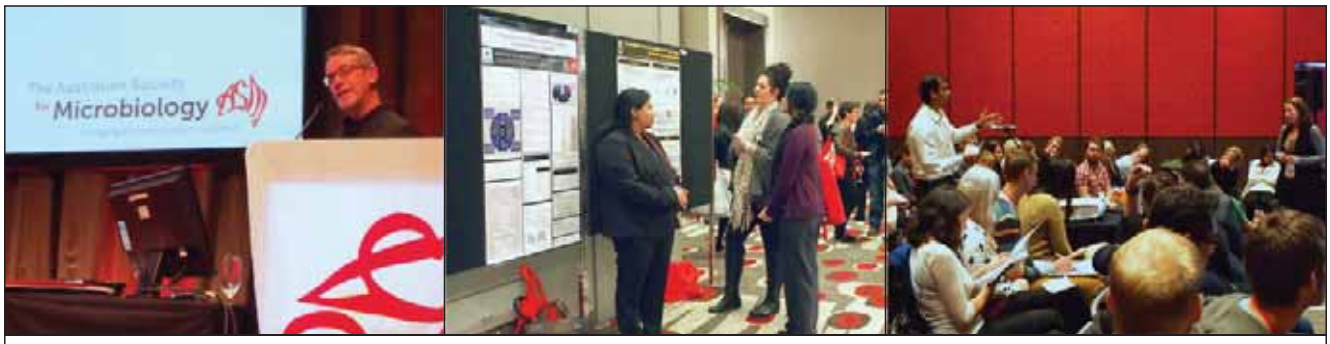

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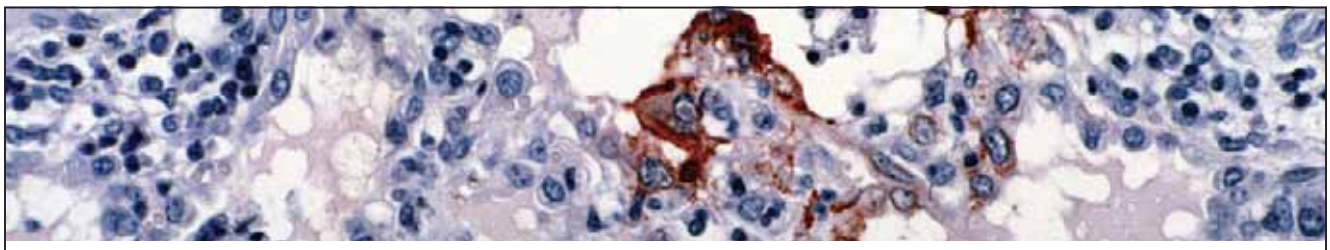

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Global surveillance and response to the threat posed by infectious diseases

Alan P. Johnson & Joanne Freedman

The international spread of infectious disease has long been recognised. As early as the 14th century, even though the microbial aetiology of communicable diseases was not understood, international travellers were kept in quarantine to prevent the spread of diseases such as the plague. In modern times, the ready availability of international air travel and other forms of rapid transport has made containing the spread of disease even more of a challenge.

To this end, International Health Regulations (IHR) were developed by the World Health Organization (WHO) in 1969 to enable countries to work together to prevent and control public health threats while at the same time trying to avoid unnecessary interference with international travel and trade. The IHR have their origin in the International Sanitary Regulations, originally devised in Paris in the mid-19th century in response to the need for international cooperation in public health following the cholera epidemics that hit Europe in 1830 and 1847.

The importance of surveillance

The development of interventions to control the spread of infectious diseases requires an understanding

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of their underlying epidemiology.

The cornerstone of epidemiology is surveillance, which comprises the collection, collation and analysis of data on the occurrence and burden of disease and the dissemination of information to those who need to know; this will include public health officials, policy-makers, healthcare professionals and the public. While many countries, at least in high-income economies, have national surveillance systems, global surveillance coupled with action to control disease spread is much more complex, requiring international cooperation and sharing of information. As an example of this, Public Health England has published its Global Health Strategy (2014–2019) as part of its commitment to improving health globally. At a wider geographical level, the European Centre for Disease Prevention and Control (ECDC) coordinates a number of pan-European surveillance networks for a range of diseases (Table 1), with responsibility for provision of national data resting with competent bodies in each country. For surveillance of antibiotic resistance, a further network known as CAESAR (Central Asian and Eastern European Surveillance of Antimicrobial Resistance), established by the WHO Regional Office for Europe, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Dutch National Institute for Public Health and the Environment (RIVM) includes all countries of the WHO European Region that are not part of the European Antimicrobial Resistance Surveillance Network (EARS-Net).


Namepic/Thinkstock

Table 1. Surveillance networks managed by the European Centre for Disease Control covering a range of infections

Disease area	Networks
Antimicrobial resistance and healthcare-associated infections	• European Antimicrobial Resistance Surveillance Network (EARS-Net) • Healthcare-associated infections Network (HAI-Net) • European Surveillance of Antimicrobial Consumption Network (ESAC-Net)
Emerging and vector-borne diseases	• Emerging Viral Diseases-Expert Laboratory Network (EVD-Net) • European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents
Food- and waterborne diseases and zoonoses	• Food- and Waterborne Diseases and Zoonoses Network (FWD-Net) • The European Creutzfeldt–Jakob Disease Surveillance Network (EuroCJD) • European Legionnaires' Disease Surveillance Network (ELDSNet)
HIV, STI and blood-borne viruses	• European Network for HIV/AIDS Surveillance • European Network for STI Surveillance • European Network for Hepatitis B and C Surveillance
Influenza and other respiratory viruses	• European influenza surveillance network (EISN)
Tuberculosis	• European tuberculosis surveillance network
Vaccine-preventable diseases and invasive bacterial infections	• EUVAC-Net (measles, rubella, mumps) • European Network for Pertussis Surveillance • European Invasive Bacterial Infections Surveillance Network (EU-IBIS) • European Diphtheria Surveillance Network (EDSN)

In 1995, a resolution was submitted to the World Health Assembly, urging all WHO Member States (MS) to strengthen surveillance and reporting of re-emerging and new infectious diseases. In response, the WHO created a global surveillance system comprising a 'network of networks' which link together existing local, regional, national and international networks of laboratories and medical centres into a super surveillance network. Participants include the WHO Collaborating Centres and Regional Offices, national and international public health bodies and centres of excellence such as the ECDC, the US Centers for Disease Control and Prevention (CDC), reporting networks of UN agencies (e.g. UNICEF) and the Training in Epidemiology and Public Health Intervention Network (TEPHINET),

which provides field epidemiology training programmes in 88 countries. Other participants include non-governmental organisations such as the Red Cross, the Red Crescent, Médecins Sans Frontières and Medical Emergency Relief International (Merlin).

Following the epidemic of severe acute respiratory syndrome (SARS) in 2003, the IHR were revised in 2005 to further strengthen surveillance and response capability. The revised IHR (2005), which are legally binding, came into force in July 2007, and represent an agreement between 196 countries, including 194 WHO MS, to build capacity to detect, assess and report public health threats in a timely manner. There is also a requirement to ensure that international ports, airports and ground crossings have the capacity to deal with public health threats and limit the spread of disease into neighbouring countries. However, in practice such activities are very resource-intensive, and in 2015, only 43% of participating countries reported having achieved full compliance.

Sharing information via the Internet

In addition to the formal surveillance activities described above, a vast amount of data is now collected and shared using the Internet, with freely available electronic discussion sites being valuable sources of information. This is particularly important for the collection of information from low-income countries, which often lack robust surveillance systems due to lack of resources and poor national infrastructure. The scope of this approach may be worldwide [e.g. ProMed (Program for Monitoring Emerging Diseases)], regional (e.g. PACNET in the Pacific region) or national (e.g. Sentiweb in France). This approach to information gathering is being increasingly used by healthcare professionals, an example being the Global Public Health Information Network (GPHIN), an electronic surveillance system developed by Health Canada which has search engines that actively trawl the Internet for reports of communicable diseases in electronic discussion groups or on news wires. GPHIN has begun to search in English and French and will eventually expand to all official languages of the WHO. Another such system is HealthMap, developed by a team at Boston Children's Hospital in 2006. Data on emerging public health threats are made freely available via the Internet at **www.healthmap.org/en** and are also available from the mobile app, Outbreaks Near Me. The aggregated data provided by HealthMap are derived from a wide range of freely available sources including, among others, ProMED, GeoSentinel (clinician-based sentinel surveillance of individual travellers), OIE (the World Organization for Animal Health [Office International des Epizooties]), FAO (Food and Agriculture Organization of the UN), Eurosurveillance and Google News. It is of note that in early 2014, HealthMap tracked early press and social media reports of a haemorrhagic fever in West Africa that was subsequently identified by WHO as Ebola.

The global response to disease

Public health threats posed by some disease outbreaks may extend beyond an affected state's national border and require coordinated international action. After convening an expert Emergency Committee, WHO may designate these as Public Health Emergencies of International Concern (PHEICs), allowing the implementation of temporary control measures. WHO may also coordinate a response using resources from the Global Outbreak Alert and Response Network (GOARN), which is

Fig. 1. **Cases of multidrug-resistant TB in Europe in 2014.** Dataset provided by ECDC based on data provided by WHO and Ministries of Health from the affected countries (data available at: **http://microb.io/2dGZOFj**).

The views and opinions of the authors expressed herein do not necessarily state or reflect those of the ECDC. The accuracy of the authors' statistical analysis and the findings they report are not the responsibility of ECDC. ECDC is not responsible for conclusions or opinions drawn from the data provided. ECDC is not responsible for the correctness of the data and for data management, data merging and data collation after provision of the data. ECDC shall not be held liable for improper or incorrect use of the data.

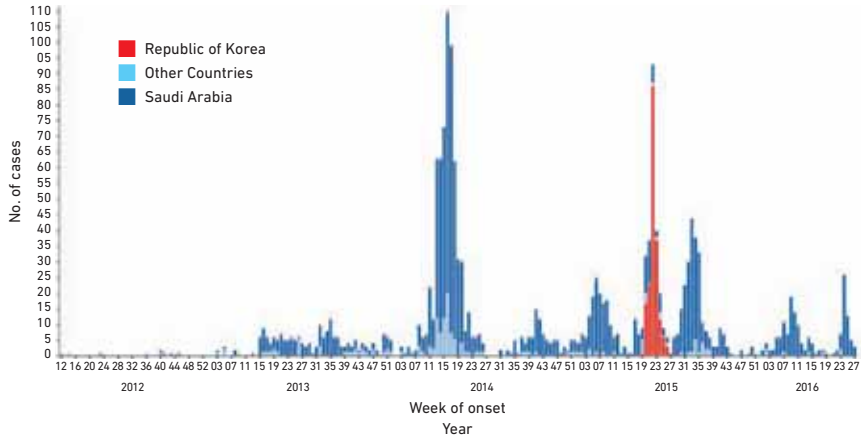

Fig. 2. Time trend for confirmed global cases of MERS-CoV reported to WHO as of 22 July 2016 (*n*=1791).
http://www.who.int/csr/disease/coronavirus_infections/maps-epicurves/en

Fig. 3. Global occurrence of Zika virus reported via HealthMap for the last week of July 2016.
www.healthmap.org

a collaboration of existing institutions and networks in different countries. A WHO team may be sent to instigate initial control measures and make an assessment of the response required. Rapid dissemination of information is important, and WHO alerts are made publically available via the Internet at **www.who.int/csr/don/en**.

Re-emerging and new infectious disease threats

Despite advances in medicine, public health continues to be threatened by re-emergence and international spread of infectious diseases thought to have previously been under control such as tuberculosis (TB), which is now increasingly difficult to treat due to multidrug resistance (Fig. 1). In addition, recent decades have seen the emergence of new infectious agents such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome coronavirus (MERS-CoV) (Fig. 2), Ebola, Zika virus and chikungunya, as well as new variants of known pathogens such as influenza virus. We have also seen the emergence of new syndromes such as microcephaly associated with the Zika virus (Fig. 3) and hantavirus pulmonary syndrome. Efforts to control the spread of these infections will require improved detection, diagnosis and reporting, as well as close international collaboration and sharing of information to trigger international responses. These will include mobilisation of healthcare personnel, and provision of clinical and travel advice aimed at mitigating the spread of disease.

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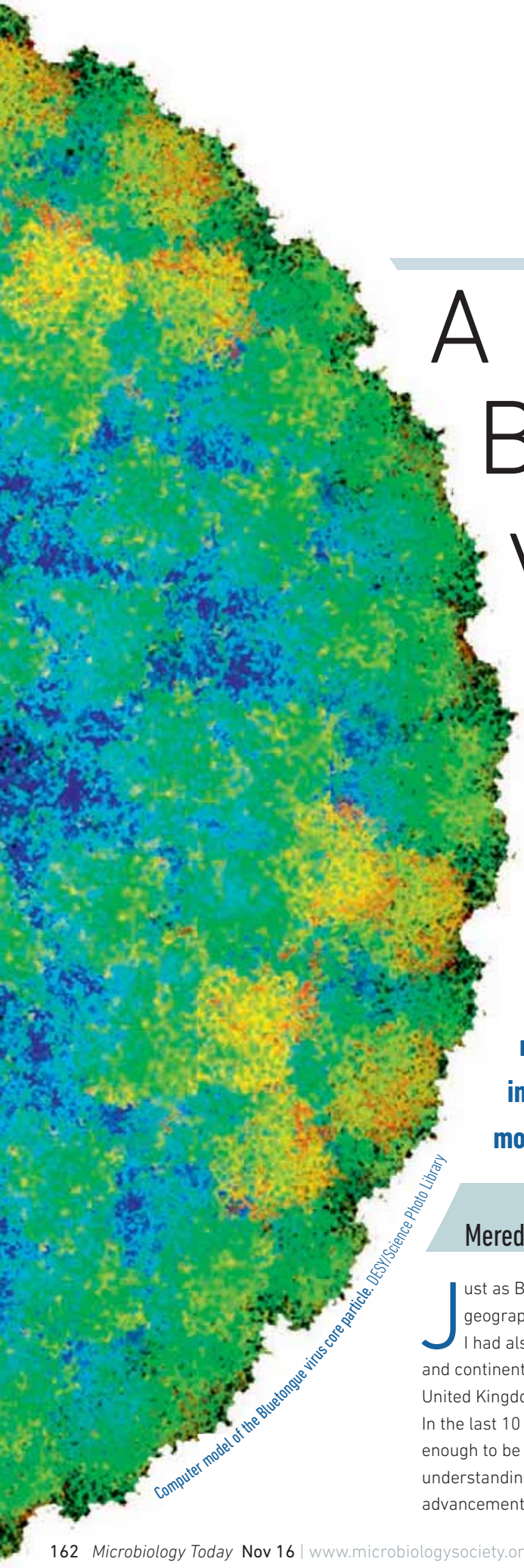

Computer model of the Bluetongue virus core particle. DESY/Science Photo Library

A spotlight on Bluetongue virus?

In the late 1990s, there were rumblings that Bluetongue virus (BTV) was on the move. The 2006 summer outbreak changed the way that the European economic and scientific communities viewed its importance. It shifted from being a neglected disease confined to the tropical regions of the world to a potentially important threat to agriculture. Suddenly, BTV was sharing research priorities and the limelight with other important viruses of animals such as foot-and mouth disease virus and avian influenza virus.

Meredith Stewart

Just as BTV had changed its geographical location in 2006, I had also moved across oceans and continents from Australia to the United Kingdom to study this virus. In the last 10 years I have been lucky enough to be involved with the increased understanding and technological advancements that have ensued due to the impact of a highly pathogenic virus entering a new environment.

The importance of BTV

BTV is a member of the *Reoviridae*, a family that includes the diarrhoea-causing rotavirus. But, unlike rotavirus, which is transmitted via the faecal–oral route, BTV transmission occurs between

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A biting midge (*Culicoides nubeculosus*) engorged with blood – a potential transmitter of Bluetongue disease.
Sinclair Stammers/Science Photo Library

ruminant animal hosts (e.g. sheep, cattle) via biting midges (*Culicoides* species) taking a blood meal. For those readers that live in Scotland or in Western Australia, you may be all too familiar with these swarming, biting insects that use you as their own personal summer smorgasbord. Classically, BTV is non-contagious; therefore, without these biting midges BTV would not be able to spread from animal to animal. But there is a newly identified strain (BTV-26) that may be transmitted via direct contact.

The clinical symptoms of Bluetongue disease depend on viral strain, host species and even animal breed. In general, sheep are the most susceptible to disease, in particular, breeds of European descent. Typically, the disease presents as an acute period of high fever (5–7 days), excessive salivation and sweating, laboured breathing, swelling of the face and, in ~10% of cases, a cyanotic (blue) tongue. Not all sheep develop clinical signs, but those that do rapidly lose condition, with the sickest generally

The emergence of BTV into northern Europe placed a spotlight on the BTV research community to rapidly respond and provide a solution.

dying within a week. Associated with the disease are severe increases to production costs, as the recovery of affected animals is slow, while high fever in sheep results in poor quality wool.

The European outbreak (2006–2008) is noteworthy as BTV displayed new characteristics. First, this novel strain, BTV-8, was exceptionally virulent, with fatalities in sheep reaching 40%. Furthermore, BTV-8 could also induce clinical signs in cattle. The virus also crossed the placenta and caused disease in the foetus; something that had only been observed with certain live BTV vaccine strains. From an epidemiological perspective, BTV-8 was being transmitted exclusively by European midge species that had not previously been shown to be capable of sustaining an outbreak.

The impact of the BTV-8 outbreak was devastating. In addition to direct losses, regions where BTV is endemic or where outbreaks occur are now subject to international trade restrictions; the economic cost of the 2007 BTV outbreak in France was $1.4 billion and $85 million in the Netherlands.

Changing locations

Historically, BTV had been confined to regions between 40° N and 35° S latitudes, including Africa, the Middle East, India, China, the United States and Mexico. Although BTV is also endemic in South-east Asia, Papua New Guinea, northern South America and northern Australia, these countries are considered to be free of clinical disease by the World Organization for Animal Health. This is in part due to the particular sheep breeds present, or a lack of sheep present in the endemic regions of these countries. In Europe, Bluetongue disease was considered exotic with sporadic cases localised to the Mediterranean Basin until the late 1990s. Although outbreaks can sometimes be attributed to animal movement, encroachment into naïve territories is primarily due to the windborne dispersal of BTV-infected midges. This allows expansion of the virus over large geographical areas (e.g. from Indonesia to Australia). In Australia BTV spread has been predicted to be due to the climatic conditions (e.g. wet, warm springs), while in Europe the size of the midge population/susceptible animal populations are critical factors. Recently, an Oxford group showed that 38% infection of BTV during 2006 was due to midges moving upwind under their own power. These findings have implications for other viruses and pathogens spread by biting midges.

The increased incidence of BTV in new environments is a clear indication that the geographical location of BTV is expanding. The source of the outbreak of BTV-8 in northern Europe is still unknown. It may have been introduced by different mechanisms other than the wind-assisted movement of infected *Culicoides* species. This includes the movement of infected livestock, use of live attenuated vaccine strains and the importation of midges with flowers or fresh produce. Furthermore, unlike the previous movements of the virus throughout Europe, BTV-8 expanded from Northern Europe (i.e. Belgium, France) into Italy and Spain, crossing the major physical barriers of the Swiss Alps and Pyrenees mountain range.

Traditionally, spread of BTV in Europe was linked to the geographical distribution of the African midge (*Culicoides imicola*), which has extended northwards as a consequence of climate change. The ability of the midge to be infected by BTV (vector competence) was always postulated to be a factor that hampered BTV's geographical spread. Infection of European midges (*Culicoides obsoletus* and *C. pulicaris*) was often associated with a lag time where other BTV strains had to 'adapt'. BTV-8 infection of the European *Culicoides* species did not display this lag time. These viral characteristics may have assisted the rapid spread throughout Europe.

Geographical change of BTV research

The emergence of BTV into northern Europe placed a spotlight on the BTV research community to rapidly respond and provide a solution. The biggest factors identified with enabling BTV to spread throughout Europe were strategies to identify and control the spreading outbreak, and the lack of suitable vaccines. The use of live attenuated vaccines in Italy had proven to be troublesome. Just as the virus had dispersed, scientists like myself were travelling through Europe as part of a collaborative research network.

Importantly, diagnostic tests with rapid turnaround times were developed. This led to the identification of new types of BTV and a system to identify all circulating strains of BTV. The advancement in vaccine development was aided by molecular tools to manipulate the virus, and protein expression tools. The research group I belonged to was involved in developing and testing new vaccines with DIVA (discriminate between vaccinated and infected animals) potential to limit the impact of trade restrictions.

This network of scientists provided a greater understanding of virus–host interactions and factors that enabled BTV outbreaks to occur (weather, midge population, etc.). Importantly, these results aided in the rapid response to another midge-transmitted virus of sheep (Schmallenberg virus) in Europe years later. Although a wealth of knowledge has been generated, there are still big questions to be tackled, with the threat of a new outbreak always present.

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Chytridiomycosis as a cause of global amphibian declines

Thomas J. Burns, Mark S. Greener & Paul A. Hoskisson

Amphibians are remarkable creatures that have inhabited the Earth for over 350 million years, and exhibit some of the most amazing and diverse life histories. The planet is home to around 7,500 species of amphibian, which occupy an extraordinary number of ecological niches. They are often viewed as indicators of environmental health by ecologists due to their reliance on both aquatic and terrestrial environments to complete their lifecycles. Furthermore, their thin and highly sensitive skin, where much of their respiration occurs, makes them highly susceptible to environmental toxins, disease and radiation.

Beginning of the decline

In the mid-1980s amphibians began to decline at an alarming rate, with a number of species being considered as extinct. Much of this decline was attributed to habitat loss, climate change and environmental pollution. At first these declines were noticed in Central America and Australia; however, mass mortality events also began to occur in what were considered to be pristine environments. In 1998 the major pathogen responsible for these declines was identified as the zoosporic nonhyphal euchytrid, *Batrachochytrium dendrobatidis*. The euchytrids are believed to be an early diverging branch within the fungal kingdom that use zoospores as the primary mode of dispersal, a trait which is believed to have been lost in the higher fungi as new spore dispersal mechanisms evolved. Moreover, it is this zoospore stage of the chytrid lifecycle that is fundamental to amphibian pathogenicity.

The lifecycle of *B. dendrobatidis* begins as an aquatic spore with a single flagellum. These zoospores disperse within the environment, where they may come into contact with the thin,

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permeable skin of amphibians. Upon contact with amphibian skin, the spores penetrate the skin and the zoospores encyst, absorbing their flagellum and forming a cell wall. Subsequently the cyst germinates, developing a small germ tube, which allows tissue and cell penetration. The fungal cells proliferate intracellularly and the germ tube gives rise to the sporangium. The infected cells are carried to the skin surface during epidermal differentiation, where the mature zoospores are released into the environment via discharge tubes. It is this process of growth and differentiation in the sensitive skin that is highly damaging to amphibians and results in the pathogenic effects. *B. dendrobatidis* is known to infect over 500 species of amphibian and has resulted in global amphibian declines. Remarkably, there is huge variation in the pathogenic effects of *B. dendrobatidis,* with some species being highly sensitive, with devastating effects on the population, and with other species appearing to be unaffected by infection and potentially acting as environmental reservoirs for *B. dendrobatidis.* Genomic studies of *B. dendrobatidis* indicate that it has a complex evolutionary history with the population structure consisting of multiple divergent lineages with no single evolutionary transition being linked to the observed global amphibian declines. This points to a multifactorial cause for global amphibian declines, perhaps linking evolutionary and ecological causes such as increased global trade of amphibians distributing *B. dendrobatidis* across the world, coupled with climate change and possibly other, as yet undiscovered, causes.

B. dendrobatidis – the first chytrid fungus

B. dendrobatidis has been found on every continent where amphibians occur (all

Above **Common frog (*Rana temporaria*).** Paul A. Hoskisson
Below **Glass frog (*Hyalinobatrachium orientale tobagoensis*).** Paul A. Hoskisson
Inset ***Batrachochytrium dendrobatidis* on the skin of an amphibian.** Dr E. Davidson, Visuals Unlimited/Science Photo Library)

except Antarctica) and linked to their decline. In Europe, *B. dendrobatidis* is widely distributed and has been linked to declines in a range of species, with midwife toads (*Alytes obstetricians*) and natterjack toads (*Epidalea calamita*) being particularly affected. More recently, the emergence of a second *Batrachochytrium* species, *B. salamandrivorans,* has resulted in huge losses in fire salamander (*Salamandra salamandra*) populations in northern Europe. It would appear that *B. salamandrivorans* emerged in Asia and has coexisted with certain species of amphibian there; however global amphibian trade has resulted in its introduction to naïve amphibian populations in Europe with devastating effects. Whilst resulting in huge losses in salamander populations, *B. salamandrivorans* has so far been restricted to urodele amphibians (newts and salamanders).

Chytrid-mediated amphibian declines in Australia (in line with the rest of the world) date back to the late 1970s, with Queensland's gastric brooding frog (*Rheobatrachus silus*) being the first species to succumb to extinction. This species declined in the winter of 1979, and was last sighted in the wild in 1981. Prior to the identification of chytrid fungus in 1998, there was much debate on the cause of such dramatic amphibian declines around the globe, especially those that occurred in apparently pristine habitat. Australia was at the forefront of this debate, with observations of declines spreading northwards up the Queensland coast leading to early (and at that time unpopular) suggestions that a disease epidemic may be the cause of declines.

Australia was initially proactive in developing policy to combat chytrid

Fire salamander (*Salamandra salamandra*). Paul A. Hoskisson

Southern corroboree frog (*Pseudophryne corroboree*). Paul A. Hoskisson

It is clear that chytrid-mediated amphibian declines are a complex problem, of which there is still much to be discovered. Understanding the disease dynamics for amphibian species which have experienced complex chytrid-mediated declines will be a substantial challenge.

fungus, listing it as a 'key threatening process' in 2002 and drawing up a Threat Abatement Plan in 2006. Recently, there have been calls for more to be done; a recent review of chytridiomycosis management and the adequacy of conservation efforts in Australia highlighted seven species at immediate risk of extinction and a further 22 species at moderate or lower risk. With Australia's 238 species, that equates to > 10% of all Australian amphibian species facing extinction risk from chytrid.

Risk of extinction

Of the seven species identified as at immediate risk of extinction, three of these - the Baw Baw frog (*Philoria frosti*), the southern corroboree (*Pseudophryne corroboree*) and northern corroboree frogs (*Pseudophryne pengilleyi*) are temperate, sub-alpine specialists from south-eastern Australia. Unlike the tropical Queensland frogs, which declined rapidly as chytrid spread, these populations declined over a period of decades. Both Baw Baws and southern corroborees experienced an initial dramatic decline in population, distribution and density in the late 1980s/early 1990s, followed by a more gradual decline since. This suggests a more complex interaction with the chytrid pathogen. So, what were the driving factors in population decline for these species? Have they changed over time and how may they interact?

All three species are relatively long-lived, slow to mature and predominantly terrestrial. They make use of both heath/bog and forest habitat, and lay their eggs in terrestrial nests within their breeding habitat, as opposed to a focal pond. These are not attributes that appear immediately conducive to the maintenance and spread of an aquatic pathogen, at least not at population densities existing in the wild today. Perhaps key to understanding their continuing decline is their co-occurrence with common eastern froglets (*Crinia signifera*), which is a non-susceptible species that may act as an environmental reservoir host of chytrid.

It is clear that chytrid-mediated amphibian declines are a complex problem, of which there is still much to be discovered. Understanding the disease dynamics for amphibian species which have experienced complex chytrid-mediated declines will be a substantial challenge, but one that will be essential for predicting and mitigating the impacts of chytrid on amphibian populations on a global scale.

Acknowledgements

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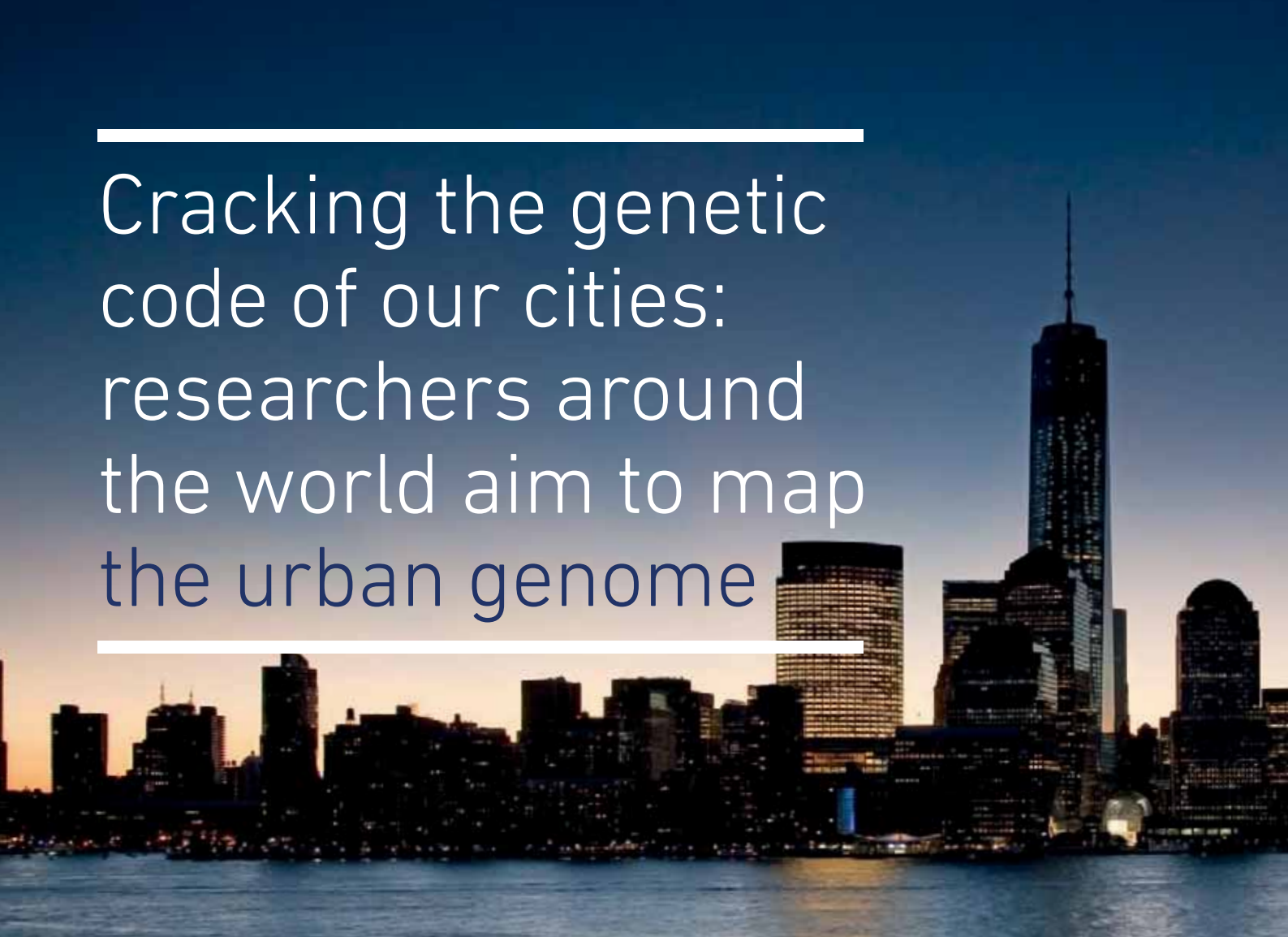


Cracking the genetic code of our cities: researchers around the world aim to map the urban genome

Sofia Ahsanuddin, Ebrahim Afshinnekoo & Christopher E. Mason

It is rare to say that one has lived through a revolution, but we are all living through one right now. High-throughput sequencing technologies have become cheaper and more cost-effective over the past decade, moving even faster than Moore's Law for computer power (doubling every 18 months). Because sequencers are modern-day 'molecular microscopes', scientists believe that we are currently experiencing a scientific revolution similar to the one sparked by Antonie van Leeuwenhoek's invention of the world's first light microscope in the 17th century.

The past few decades have witnessed a surge in microbiome and metagenomics studies, all of which are intent on elucidating the vast invisible world beneath our fingertips. With the advent of next-generation sequencing technologies, we are able to study this world like never before.

The birth of PathoMap

In 2010, Dr Christopher Mason, Associate Professor in the Department of Physiology and Biophysics at Weill Cornell Medicine, was suddenly struck by inspiration as he watched his daughter play with toys with her friends on the train. The infants unknowingly exchanged bacteria with one another as they drooled on their toys, passing them from hand to hand, and rolling them on

Reproduced from *Microbiology Today*, with permission.

New York. Caia Image/Science Photo Library

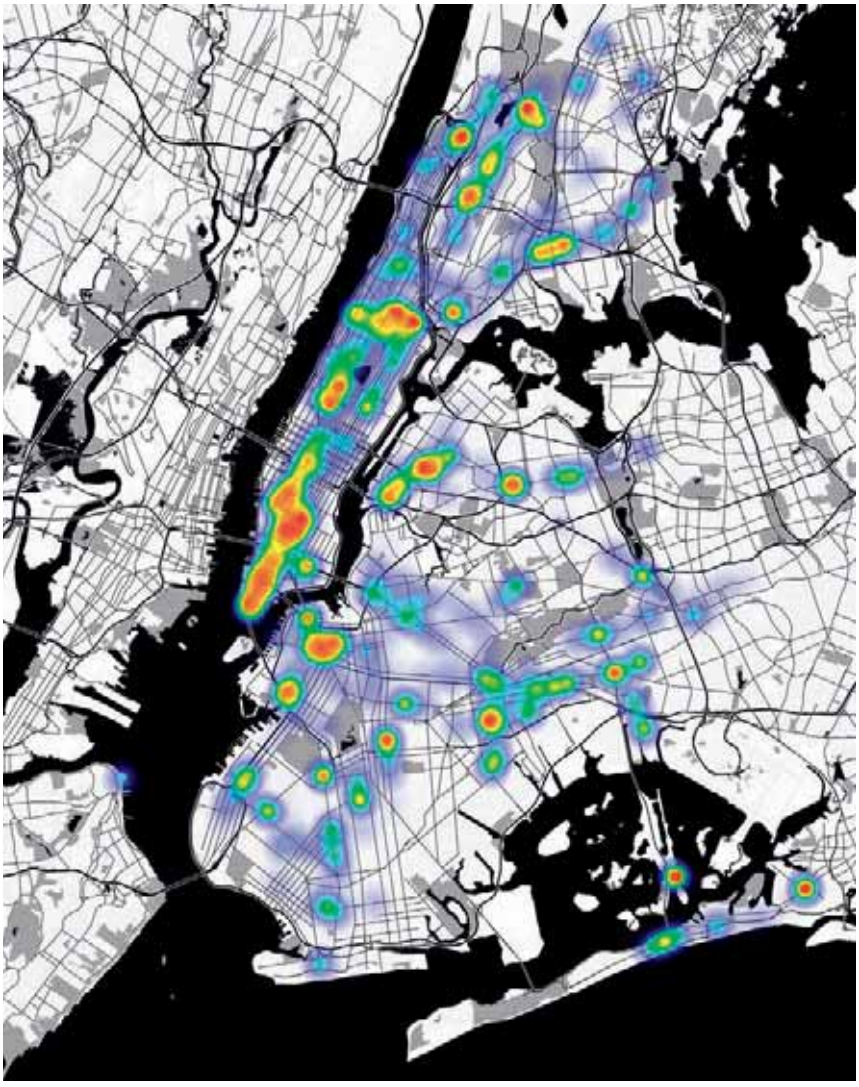
the ground before repeating the cycle. These observations eventually gave birth to the PathoMap (**www.pathomap.org**) study in 2013, in which Dr Mason and his research team created a molecular portrait of the New York City subway system. Inspired by Dr Mason's daughter, the PathoMap study aimed to pioneer the field of metagenomics (study of all kingdoms' environmental DNA) and microbiome studies by creating the first baseline geospatial metagenomic map of a city's mass-transit system.

The PathoMap study's primary objective was to study the microbiome of a metropolitan environment. Since New York City's subway is the most highly trafficked transit system in the United States, it made perfect sense to sample all 468 stations to investigate the genetic and microbial diversity present in each station. A team of volunteers collected and processed a total of 1,547 samples. Using next-generation sequencing technologies and bioinformatics to analyse the sequencing data, the research team was able to determine the taxonomic classification and functional diversity of the micro-organisms present on such ubiquitous surfaces as handrails, wooden benches, train seats, rubbish bins and floors. Most of the DNA uncovered from these surfaces matched bacteria associated with the skin microbiome. Altogether, the researchers found over 600 species of microbes riding the subway with fellow New Yorkers. Furthermore, while the research team found evidence of antibiotic resistance markers and antibiotic resistant microbes, there was no evidence of virulence factors or pathogenicity in the samples. Interestingly, nearly half (48%) the DNA matched no known organism, which is a testament to the fact that we are only as good as our databases. As the field develops, our databases will become more refined and replete with accurate references that may impact the finality of taxonomic classification at species- and strain-levels.

PathoMap's implications for human health, illness and disease

Creating the world's first-ever metagenomics profile at the city-scale has tremendous implications for the future of public health and epidemiology. For one thing, it is the first step to creating futuristic real-time pathogen monitoring systems in urban spaces to prevent the rapid spread of epidemic and pandemic-scale disease outbreaks. The study may also help urban planners and engineers better utilise microbial ecology to design sustainable and healthy cities. Specifically, this study opens the door to incorporating the microbial world in our understanding of how building materials complement the urban microbiome. We are unknowingly building urban microbiomes each time we construct a new building or renovate a space. Studies like the PathoMap study suggest that it is crucial to incorporate a comprehensive understanding of the microbiome in order to improve upon environmental and human health. The research team was able to discover bacteria that digest toxic sludge, which may potentially help city planners and researchers formulate sustainable methods of revitalising 'Superfund' sites like the Gowanus Canal in Brooklyn, New York.

MetaSUB Global Sample Collection Map. For full interactive features, please visit **www.metasub.org/interactive-map**. The MetaSUB International Consortium and Landscape Metrics



Geospatial map of Enterobacteriaceae. Afshinnekoo *et al.*, 2015

The PathoMap study also provides a unique model of data collection and processing to the emerging field of participatory disease surveillance, whereby community members themselves report on illnesses that emerge in close proximity to them. The use of citizen science and crowdsourcing models has further closed the gap between science and society. Dr Mason and his colleagues believe that PathoMap is a testament to the power and potential of publically engaged scientific initiatives.

Inauguration of the MetaSUB International Consortium

The success of PathoMap led to the expansion of the project to other cities like Buenos Aires, Argentina; Tokyo, Japan; Cairo, Egypt; Lima, Peru; and Paris, France. In 2015, Dr Mason inaugurated the Metagenomics and Metadesign of Subways and Urban Biomes (MetaSUB) International Consortium (**www.metasub.org**) in an effort to create the world's first-ever longitudinal metagenomics profile of cities around the world. Since then, it has grown to include over 58 cities across 32 countries. Scott Tighe, one of MetaSUB's premier contributing members on developing extraction techniques for the consortium, said, "The scientific reward of benchmarking the microbial DNA content of global urban environments is an awesome undertaking." Different cities are profiling environments other than those found in the transit systems – Vienna has sampled the Danube Canal, Montevideo in Uruguay has sampled the city's beaches (MetaBEA) and sewage (MetaSEW) systems, and Tokyo plans to sample the city and university buildings. Gaston Gonnet, the Principal Investigator of MetaSEW and MetaBEA in Montevideo, remarks that "these types of projects are quite novel and the

possibility of exchanging information is very beneficial; it gives us new ideas and saves time and false starts."

The MetaSUB Consortium has so far hosted two international meetings – one in New York City, USA, and the second in Shanghai, China – where collaborators discussed the latest updates in metagenomics research and standardised experimental protocols. Moreover, consortium members participated in 'Global City Sampling Day (CSD)' on 21 June 2016 in concert with Ocean Sampling Day (**https://www.microb3.eu/osd**) to map genetic and epigenetic stratification of antimicrobial resistance markers in the urban environment. Dr Leming Shi, co-organiser of the 2nd Annual MetaSUB Summit in Shanghai, commented on MetaSUB's unique model of scientific collaboration. He said, "What impressed me the most is MetaSUB's capability of engaging a mix of well-accomplished scientists, young college students, and graduate students under the same roof with the same objective of gaining a better understanding of ourselves by better understanding the environment we live in."

Mapping human ancestral data in MetaSUB Sheffield, United Kingdom

In Sheffield, UK, Dr Eran Elhaik is working hard to piece together human ancestry data from metagenomic data found on public surfaces. Elhaik states: "One of the greatest difficulties in studying microbial ecology is their complex relationships with human populations. Inferring the geographical origins of humans from the genetic data collated during the swabbing process allows identification of the demographic forces that shaped the microbial communities. However, despite its great promise, due to technological difficulties, we are only capable of studying the combined DNA of individuals or rather 'communal DNA.' Afshinnekoo & others already showed that when classifying this 'communal DNA' to four ethnicities, their proportions are correlated with the ethnicities in the Census data. We extended this approach by classifying the 'communal DNA' to twelve potential gene pools and applying the GPS algorithm, shown to infer geographical origins with high sensitivity (0.75) and specificity (0.99) (Elhaik *et al.* 2014). Compacting the 12 gene pools to four and applying GPS to the NYC subway data yields results that are in agreement with those of Afshinnekoo & others and the Census data. Our approach can thereby be applied to infer temporal population dynamics and study their effect on micro-organism communities."

Exploring microbial diversity in MetaSUB Sydney, Australia

Rather than focusing on human ancestral data, Dr Aaron Darling and Dr Catherine Burke of the University of Technology Sydney, Australia, are focusing their efforts on delineating the taxonomic classification and functional diversity of microbes in the Sydney transit system. Dr Burke emphasises that her interests lie in exploring the effect of natural sources of air ventilation and exposure on microbial diversity in built environments because they are further correlated with positive health outcomes, like a decreased risk of asthma. Because Sydney's city train stations are exposed to a variety of different environments, she and Dr Darling are interested in seeing the effect of these different exposures on the microbiome of each station, such as outdoors vs underground, and harbour vs further inland train stations. She states, "Knowing how exposure to different environments affects microbial diversity in these public spaces could help inform their future design."

The 2016 Olympiome

Along with the launch of Global CSD, MetaSUB is launching the world's first 'Olympiome'. Co-organised by Drs Emmanuel Dias-Neto, Milton Ozório Moraes, Fernanda Kehdy, and Christopher Mason, the researchers profiled Rio's subway and other public areas before, during, and after the 2016 Rio Olympics. This initiative will better reveal how migration at large-scale public events impacts the microbiome and elucidate the genetic signatures that move between cities. Dr Dias-Neto said of the Olympiome project that it is 'a very exciting project' because it is the "first time the microbiome will be studied in a big global mass event."

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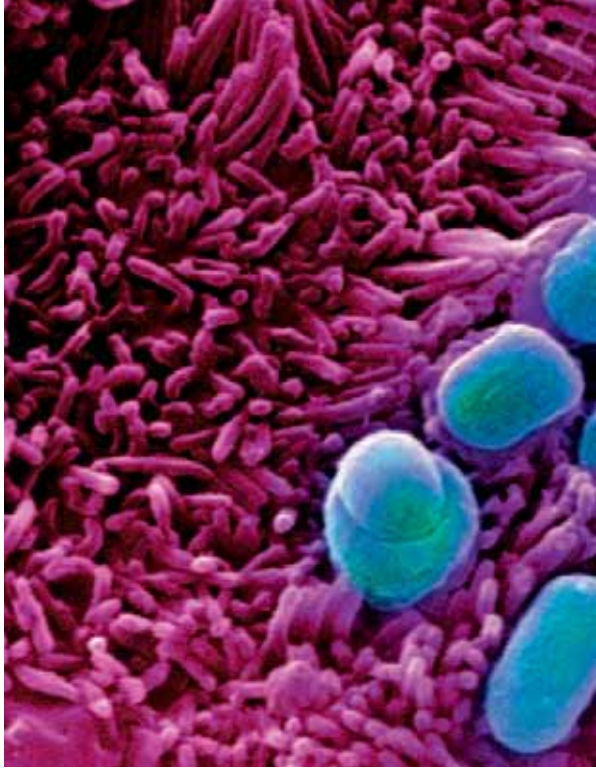
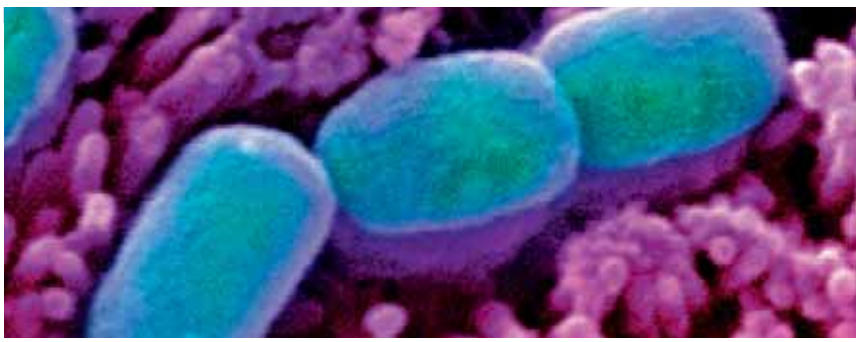
Globalisation of antibiotic resistance

David M. Livermore



Coloured scanning electron micrograph of *Escherichia coli* (blue) taken from the small intestine of a child. Stephanie Schuller/SPL

Travel always spreads disease. Bubonic plague reached Turkey in 1347 via the Silk Road, following an outbreak in 1330s China. By 1348, it raged in Italy, shadowing the gaiety of Boccaccio's *Decameron*. By 1351, half of Europe lay in plague pits. One hundred and fifty years later, the conquistadors took smallpox to the Americas, decimating local populations. They returned – many believe – with syphilis, which 'enjoyed' its first European outbreak in 1495 among Charles VIII's army, then besieging Naples. The French called it the 'Neapolitan disease' and carried it home. In England, it became the 'French pox' and in Tahiti, the 'British disease', imported by the Royal Navy.



Exponentially growing air travel (Fig. 1) accelerates the spread of bacteria. Travellers sample the local microflora – often more resistant than at home – as they eat, drink and swim, returning home colonised. What are often picked up are *Escherichia coli* with extended-spectrum β-lactamases (ESBLs), which confer resistance to modern cephalosporins. Population carriage of these is much higher in Asia and the Middle East than in Europe, Australasia or North America (Fig. 2). There is also circulation – particularly in India, but also Brazil – of bacteria with 'carbapenemases', which are β-lactamases able to hydrolyse the last-reserve β-lactams. These include the KPC, OXA-48/181 and NDM enzymes. Producers are often resistant to all 'good' antibiotics.

Cross-continent colonisation

Colonising bacteria cross continents in the guts of returning travellers. Tangden and colleagues took rectal swabs from 105 Swedish volunteers, about to travel internationally, finding just one already with ESBL *E. coli*. One hundred of the remaining 104 provided a second swab on return, and 24 became colonised,

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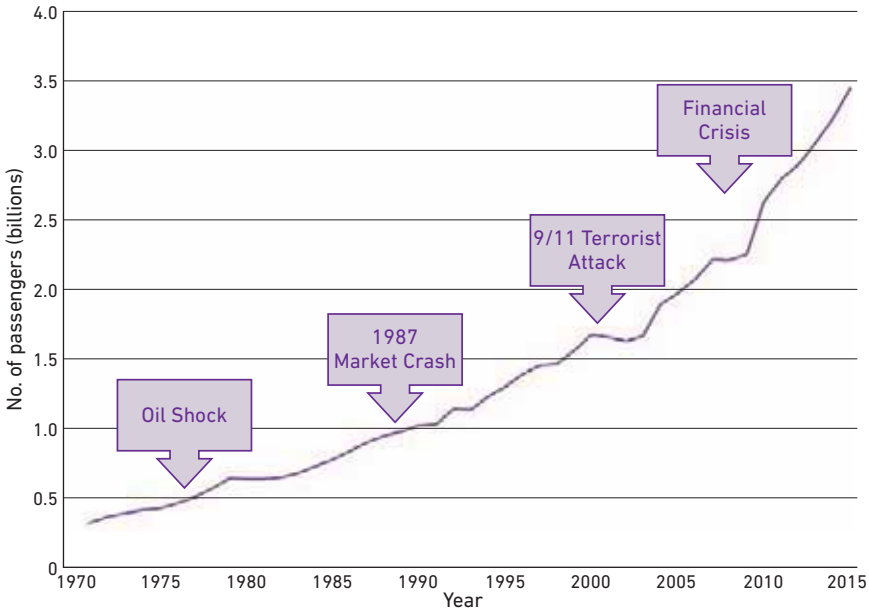

Fig. 1. **The growth of passenger air travel.** D. Livermore

including seven of eight who'd travelled to India and 10 of 34 who'd gone to East Asia. The ESBLs found were types prevalent in the countries visited – CTX-M-15 from India, CTX-M-14 from East Asia. Twenty-one of the 24 returned (!) for a third swab six months later and five remained colonised. Other studies have obtained similar results, and a meta-analysis found that travellers to South Asia stood an 88% chance of acquiring multi-resistant *Enterobacteriaceae*, increased by antibiotic use or diarrhoea, which disturb the gut flora. One-way travellers bring resistant bacteria too: 35% of unaccompanied migrant children screened in Germany late in 2015 carried ESBL *E. coli*. Colonisation by carbapenemase producers is rarer, but occurred in 3/824 travellers from France, all visiting India. In a different surveillance approach, Petersen & others sampled the toilets of aeroplanes arriving in Denmark, finding the lowest resistance gene burden in flights from Greenland and highest in those from the South and East Asia (Fig. 3).

Colonisation is innocuous if the *E. coli* remains intestinal, but the gut seeds urinary and intra-abdominal infections. Pitout, in Canada, found that infections with cephalosporin-resistant *E. coli* mostly involved strains with ESBLs prevalent in countries visited.

Some travellers are hospitalised overseas following accident or illness;

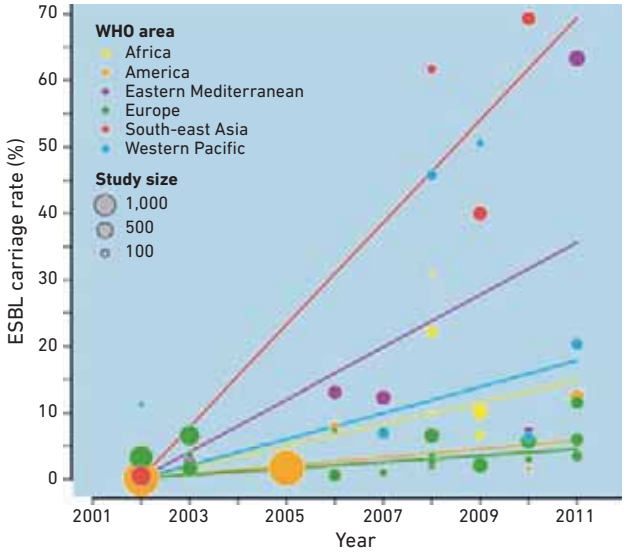

Fig. 2. **Gut carriage rates of ESBL-producing *E. coli* by time and place.** Reproduced from Woerther *et al.* (*Clin Microbiol Rev*, doi:10.1128/CMR.00023-13)

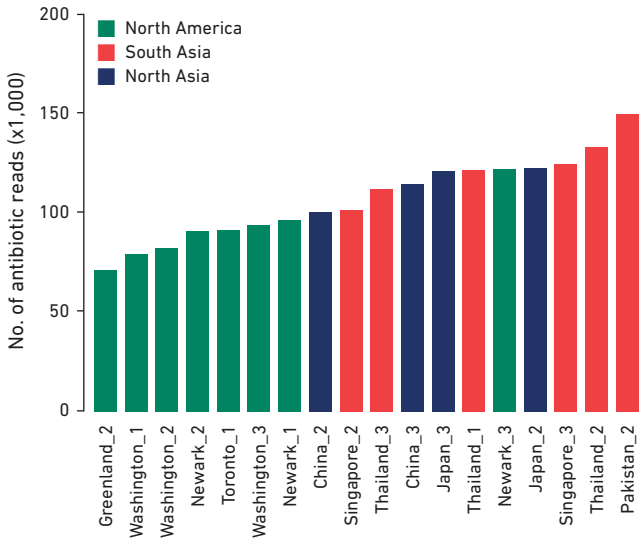

Fig. 3. **Burden of resistance genes in toilet waste from aeroplanes arriving in Denmark.** Reproduced from Petersen *et al.* (*Sci Rep* **5**, doi:10.1038/srep11444)

others travel specifically seeking medical services. Elderly UK residents with family ties to India or Pakistan divide lives and healthcare between countries. Middle-Eastern patients come to private London hospitals, whilst 'corporate' hospitals in India draw patients from Europe, Africa and the Middle East. In 2010, 63,000 UK residents travelled abroad for treatment including many to India (Fig. 4), which, in total, drew 1.27 million medical tourists in 2012, generating $1.8 billion in revenue.

Whilst many of the hospitals attended by medical tourists and accident victims provide excellent care, the fact remains that vulnerable patients are moving between low- and high-resistance countries. Moreover, by their nature, hospitals concentrate antibiotic selection pressure. At a medical college in northern India, 50% of intensive care patients were colonised with ESBL producers and 3% with carbapenemase producers. At Rawalpindi, Pakistan, 27% of inpatients and 17% of outpatients were colonised by *Enterobacteriaceae* with NDM ('New Delhi Metallo') carbapenemase in 2010.

NDM ('New Delhi Metallo') carbapenemase

NDM carbapenemases are interesting because we saw their early globalisation. They were first recognised in Sweden in 2008, carried by urinary *Klebsiella* and gut *E. coli* from a patient transferred a day earlier from New Delhi, India. In the subsequent 20 months, Public Health England received 29 *Enterobacteriaceae*, of multiple species, with NDM enzymes. These were from 25 patients, at least 17 of whom had visited the Indian subcontinent and 13 been hospitalised there, for reasons ranging from a road traffic accident to kidney transplants (Pakistan) to 'tummy tuck'. Most were susceptible only to colistin and tigecycline. Publication was followed by a controversy in the Indian press about the enzyme's name and a flurry of further international reports, many again describing patients who had visited the Indian subcontinent (Fig. 5). Reviewing the first 250 UK patients in 2013, we obtained a travel history for 100, finding half with travel to the subcontinent. Nevertheless the link is weakening: Public Health England now regularly sees NDM isolates from UK care home residents with no travel history, and there have been a few outbreaks in UK hospitals. Imported resistance is changing to low-level endemic...

Occasionally, a single import allows sharp expansion. *Enterobacteriaceae* with OXA-48 carbapenemase entered several European countries with casualties from the Libyan 'Emergency' of 2011. One became the index patient of an outbreak in a UK intensive care unit. Even starker was a Colombian patient who received a liver transplant in Israel, acquiring a sequence type (ST)258 *Klebsiella pneumoniae* with KPC carbapenemase. Back in Medellin he became the index case for a hospital outbreak, with 32 patients infected and 52 colonised.

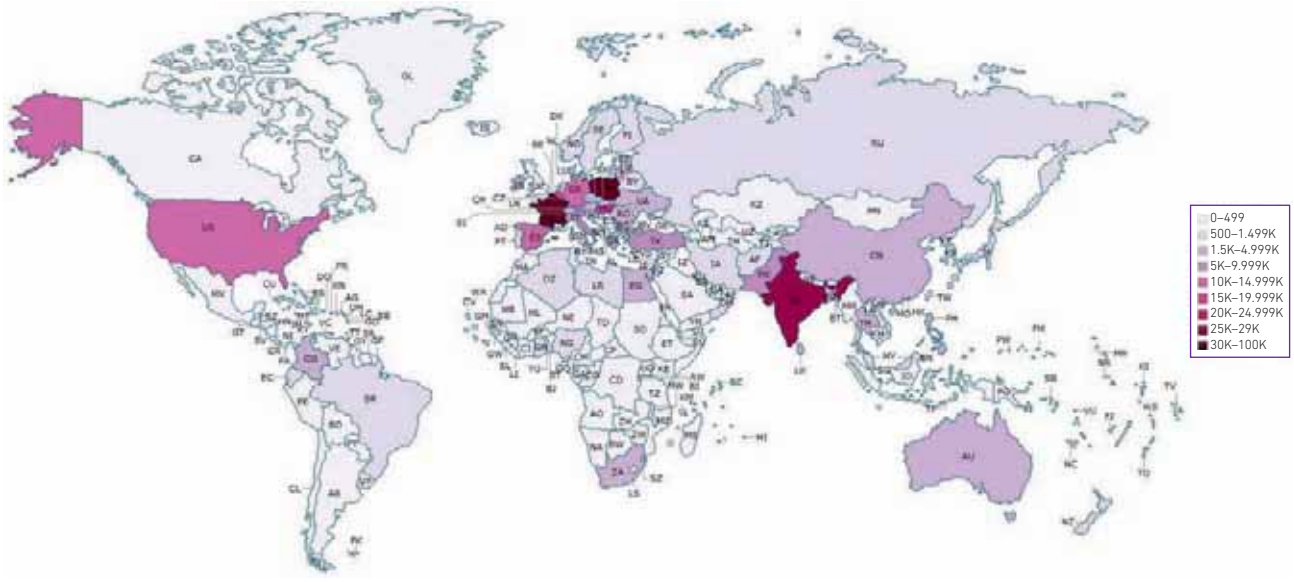

Fig. 4. **Who went where from the UK for hospital treatment, 2001–2010.** Reproduced from Hanefeld *et al.* (*PLOS ONE*, doi:10.1371/journal.pone.0070406)

Fig. 5. **Reported cases of patients with NDM *Enterobacteriaceae*, 2008–2012. Dots coloured red indicate that at least some cases have epidemiological links to the Indian subcontinent.** Adapted from Johnson & Woodford (*J Med Microbiol* **62**, 499–513, doi:10.1099/jmm.0.052555-0)

Fig. 6. **Countries where ST258 and related *K. pneumoniae* with KPC carbapenemases have been reported.** D. Livermore

KPC carbapenemases have a strong association with *K. pneumoniae* ST258 and its variants (Fig. 6), and the global spread of this combination of 'high-risk clone' and resistance likely reflects successive transfer events, as from Israel to Colombia, though most remain undocumented. Other high-risk clones have globalised too, notably *E. coli* ST131 with CTX-M-15 ESBL, but its story is more complicated as, unlike ST258 *K. pneumoniae*, this lineage often occurs without ESBLs and occasionally acquires different ESBLs besides CTX-M-15.

What can be done?

Can the globalisation of resistance be halted? The simple answer is 'No'. You can't stop people travelling or quarantine them. There is no reliable decolonisation strategy, and the duration of carriage is variable. Travellers who drank only bottled water and followed scrupulous hand hygiene were as likely to acquire ESBL *E. coli* as those who omitted these precautions, doubtless because others, who were less fastidious, prepared their food. Nevertheless, two approaches should be encouraged. First, it is vital to encourage countries with high antibiotic resistance to improve sanitation, reducing the circulation of resistant bacteria, and also to improve antibiotic stewardship and hospital infection control. The main beneficiaries will be for the local population, but the traveller will gain too. At the same time, healthcare providers in developed countries should recognise patients with a history of travel to high-resistance countries, when they are being admitted to hospitals, adapting empirical treatments and infection control precautions until the patient is confirmed *not* to be carrying unusually resistant bacteria.

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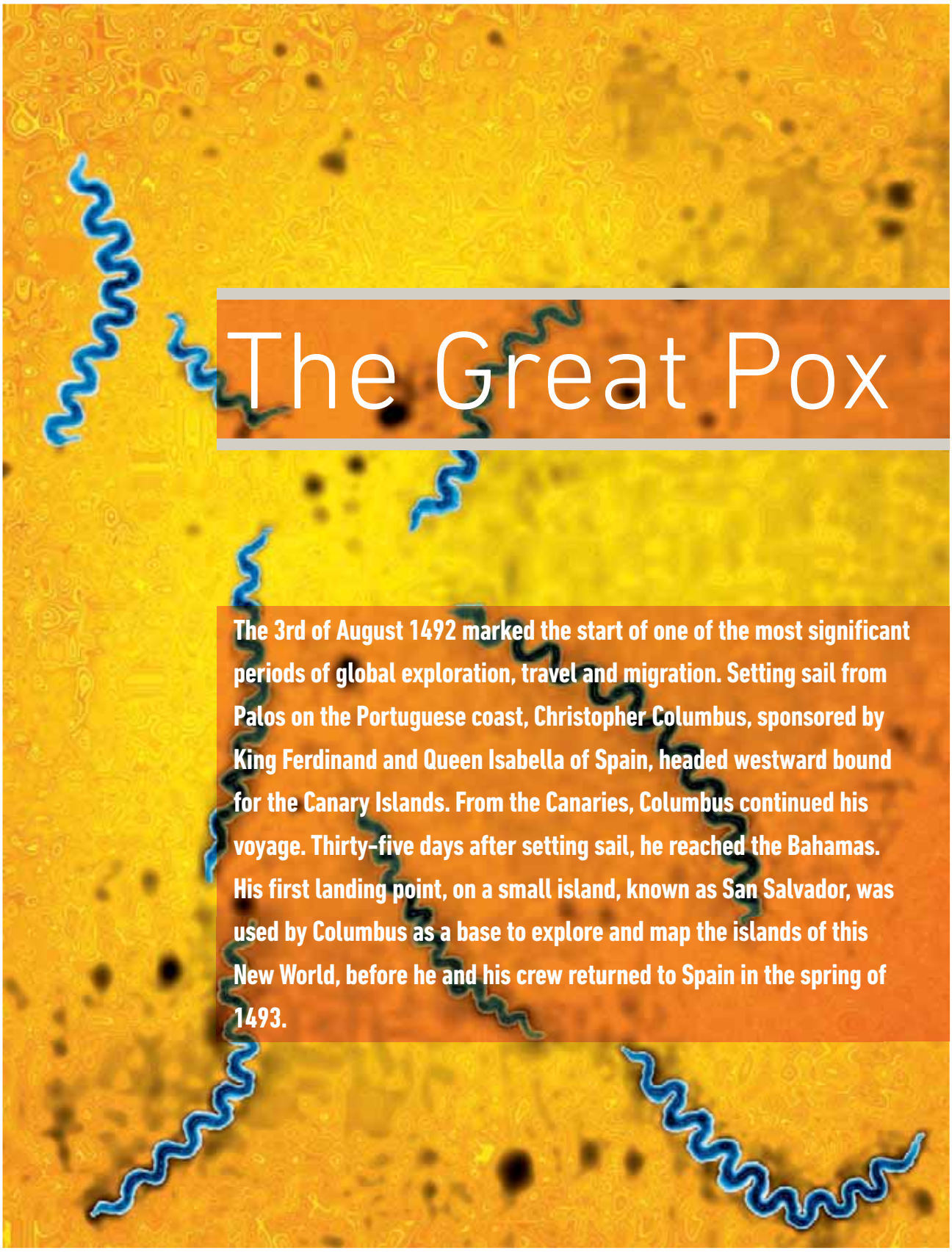


The Great Pox

The 3rd of August 1492 marked the start of one of the most significant periods of global exploration, travel and migration. Setting sail from Palos on the Portuguese coast, Christopher Columbus, sponsored by King Ferdinand and Queen Isabella of Spain, headed westward bound for the Canary Islands. From the Canaries, Columbus continued his voyage. Thirty-five days after setting sail, he reached the Bahamas. His first landing point, on a small island, known as San Salvador, was used by Columbus as a base to explore and map the islands of this New World, before he and his crew returned to Spain in the spring of 1493.

Colourised light micrograph of *Treponema pallidum*. James Cavallini/Science Photo Library

Laura Bowater

Reproduced from *Microbiology Today*, with permission.

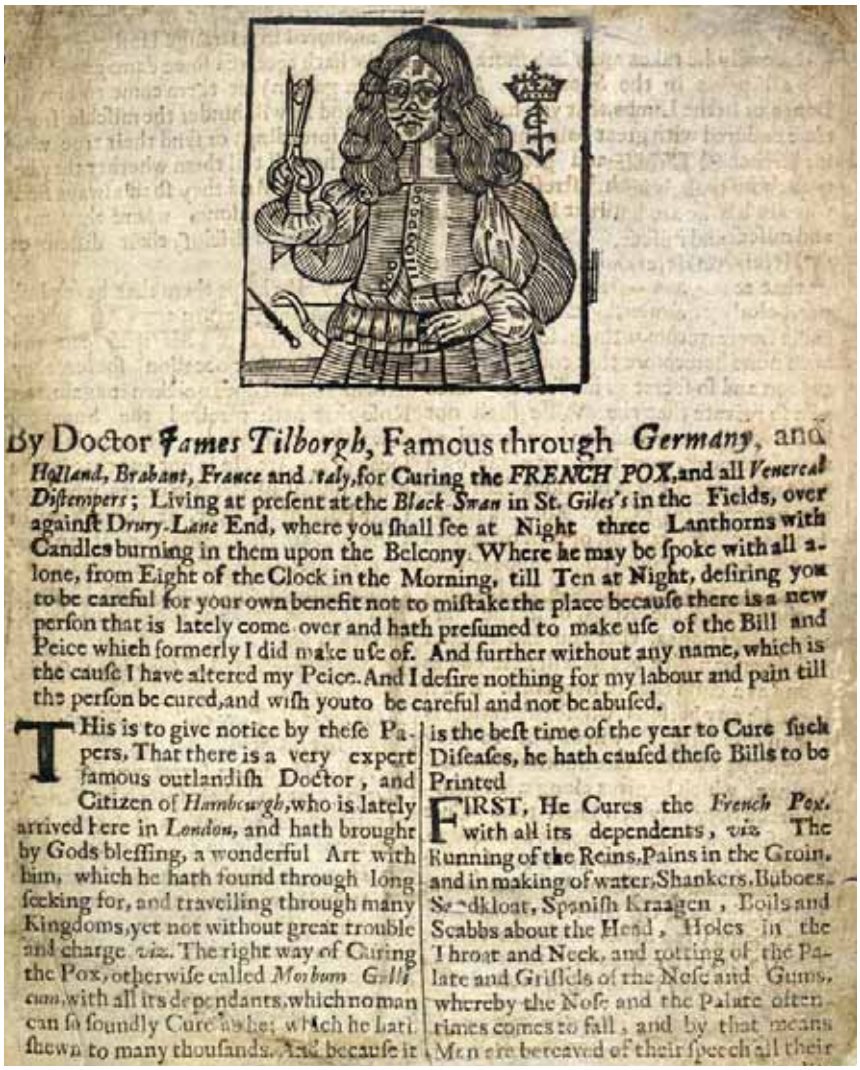
As we still see today, large migrations of human populations are often accompanied by devastating outbreaks of disease. Over time, isolated populations can build up specific immunity patterns to indigenous diseases but they are often susceptible to new infections. Columbus's exploration of this new world was no exception. Shortly after his crew's arrival, the indigenous population was decimated by epidemics of influenza and smallpox that swept across the continent. The evidence suggests that this was a mutual disease exchange; by 1495, Columbus and his crew arrived back in Europe and they brought the 'Great Pox' (as opposed to the 'Small Pox') with them. This 'Great Pox' soon gained notoriety because of the severity and location of its physical symptoms:

> *"boils that stood out like acorns, from whence issued such filthy stinking matter that who so ever came within the scent, believed himself infected"*
> [Von Hutten (1519), translation from Major (1945) p31(5)].

Today we know this disease as syphilis thanks to Girolamo Fracastoro, the famous 16th century mathematician, physician and poet from Verona, who described a dreadful plague sent by a vengeful sun god to strike down the mythical shepherd Syphilis in his poem *Syphilis sive morbus gallicus*. This name has stuck to this day.

The Age of Discovery

Europe in 1495 was mid-Renaissance and experiencing a resurgence of literature, art, sculpture and architecture. But this was also a time of turmoil and change. Shortly after Columbus returned to Europe, the French troops



By Doctor *James Tilborgh*, Famous through *Germany*, and *Holland*, *Brabant*, *France* and *Italy*, for Curing the *FRENCH POX*, and all *Venereal Diſtempers*; Living at preſent at the *Black Swan* in St. *Giles's* in the Fields, over againſt *Drury-Lane* End, where you ſhall ſee at Night three Lanthorns with Candles burning in them upon the Belcony. Where he may be ſpoke with all alone, from Eight of the Clock in the Morning, till Ten at Night, deſiring you to be careful for your own benefit not to miſtake the place becauſe there is a new perſon that is lately come over and hath preſumed to make uſe of the Bill and Peice which formerly I did make uſe of. And further without any name, which is the cauſe I have altered my Peice. And I deſire nothing for my labour and pain till the perſon be cured, and wiſh you to be careful and not be abuſed.

THis is to give notice by theſe Papers, That there is a very expert famous outlandiſh Doctor, and Citizen of *Hamburgh*, who is lately arrived here in *London*, and hath brought by Gods bleſſing, a wonderful Art with him, which he hath found through long ſeeking for, and travelling through many Kingdoms, yet not without great trouble and charge, *viz.* The right way of Curing the Pox, otherwiſe called *Morbus Gallicus*, with all its dependants, which no man can ſo ſoundly Cure as he; which he hath ſhewn to many thouſands. And becauſe it is the beſt time of the year to Cure ſuch Diſeaſes, he hath cauſed theſe Bills to be Printed

FIRST, He Cures the *French Pox*, with all its dependents, *viz.* The Running of the Reins, Pains in the Groin, and in making of water, Shankers, Buboes, Sendkloat, Spaniſh Kraagen, Boils and Scabbs about the Head, Holes in the Throat and Neck, and rotting of the Palate and Griſtels of the Noſe and Gums, whereby the Noſe and the Palate oftentimes comes to fall, and by that means Men are bereaved of their ſpeech all their

A 17th-century handbill advertising a cure for syphilis (here called the 'French Pox') and other venereal (sexually transmitted) diseases. British Library/Science Photo Library

of King Charles VIII were marching to besiege Naples in order to create a Mediterranean base to launch a Crusade. This was the start of the First Italian War. Soldiers and mercenaries were recruited from across Europe, along with more than 800 camp followers. It wasn't long before the great pox emerged within their ranks. This 'French disease', as the great pox was soon rebranded, spread remorselessly across a wide swathe of the European continent. Recognising no borders and travelling eastwards into India, China and Japan, and south into the African continent, it collected several new names along the way. These names had one thing in common – an inherent desire to attribute this terrible disease to foreigners and aliens. The French named it the 'Neapolitan disease', the Russians the 'Polish disease', the Polish and the Persians called it the 'Turkish disease', and the Turkish called it the 'Christian disease'. Further afield,

the Tahitians named it the 'British disease' and in Japan it was known as the 'Chinese pox'.

Syphilis: the bacterial disease

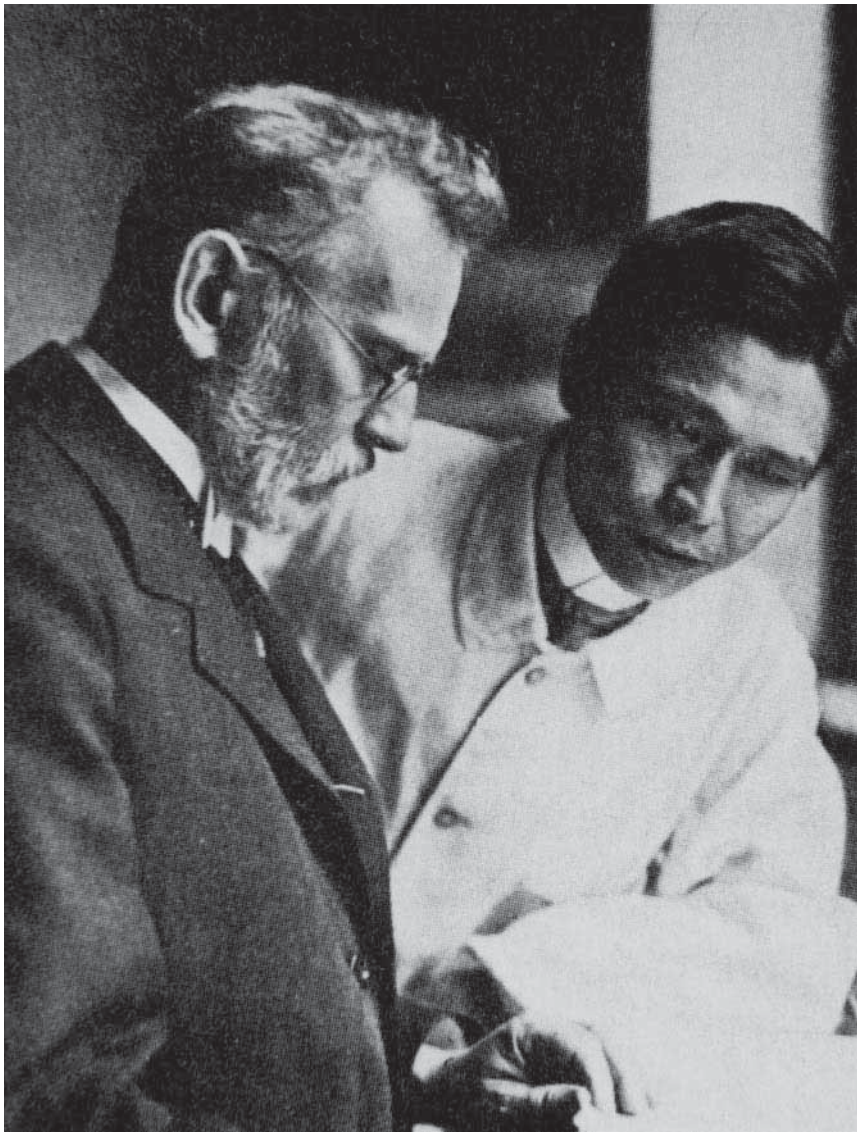
Syphilis is a sexually transmitted infection; the more sexual partners an individual has, the more likely they are to catch the disease. In the pre-antibiotic era syphilis was an extremely common disease that ravaged populations. In 1905, more than 400 years after the disease arrived in Europe, two German scientists, the zoologist Fritz Schaudinn and the dermatologist Erich Hoffmann, finally identified the bacterial agent responsible for this devastating disease. *Treponema pallidum* is a spirochaete, a delicate corkscrew-shaped bacterium that enters the body through micro-traumas and abrasions in mucous membranes.

The disease erupts in three stages. Primary syphilis, the first stage of the disease, manifests as a 'chancre' (or ulcer) appearing at the initial site of the bacterial infection. Left to its own devices, this ulcer usually heals, but unfortunately this isn't the end of the infection; the disease re-emerges as secondary syphilis. The infected individual begins to feel unwell with a fever, a rash, and a sore throat. Once again, these symptoms can appear to improve spontaneously but they can be relapsing until finally the disease retreats, becoming latent and asymptomatic. Syphilis can lie latent and hidden for many years before emerging once again as tertiary or late syphilis. It is at this stage of the disease that the most severe symptoms appear. Syphilis damages the heart, causes gummy tumours that can appear in any body tissue including the bones, and causes neurological damage that can lead to mobility problems, dementia and insanity.

Syphilis can also be passed from mother to child. A pregnant woman infected with *T. pallidum* can transmit syphilis to her foetus via the placenta, and in a third of cases this can cause stillbirth and miscarriage. Infected mothers can also give birth to babies with congenital syphilis – a severe, disabling, and often life-threatening infection.

Syphilis treatment and cures: past, present and future

In 1516, Ulrich von Hutton, a German scholar plagued with syphilis, described one of the first treatments for this disease in his poem, *De Morbo Gallico*. Guaiacum, or *holy wood*, was brought from Central America to Europe in order to treat this noxious disease. It was not an effective cure, and alternative



Portrait of Paul Ehrlich (1854–1915) and Sahachirō Hata (1873–1938), the bacteriologists from Germany and Japan, respectively, who discovered the first cure for syphilis. Science Photo Library

In the UK, the numbers of infectious syphilis diagnoses are at the highest since the mid-1950s and this pattern is repeated on a global scale.

treatments such as sweat baths and mercury ointments and inhalations (sometimes both) soon became an accepted treatment. Although mercury had terrible side effects and many patients died of mercury poisoning, it remained the go-to drug for syphilis until 1910, when Paul Ehrlich, a German physician and Nobel prize-winning scientist, discovered the antisyphilitic effects of arsenic compounds. Ehrlich's approach to treating infectious diseases like syphilis was radical. Instead of focusing on ameliorating the symptoms of syphilis, he decided to target the disease-causing agent, *T. pallidum*, curing the patient and the patient's symptoms in the process. Ehrlich and his assistant, Sahachirō Hata, a Japanese bacteriologist, began to search for a '*magische Kugel*': a 'magic bullet'. They focused on screening a raft of arsenic-based synthetic dyes by methodically testing the compounds on syphilis-infected mice. Compound 606 soon emerged as a clear frontrunner and it was quickly marketed globally as Salvarsan™ and later, the improved Neosalvarsan™. Was Salversan™ the magic bullet that Ehrlich had hoped for? Well not quite; although it effectively destroys *T. pallidum*, the drug's harmful side effects and complex treatment regime were significant issues. Eventually, a new treatment for syphilis emerged following the discovery of penicillin by Alexander Fleming in London, in 1928. By 1943, the production of penicillin had mostly moved to the United States. It was against the backdrop of mass migration caused by the Second World War that John F. Mahoney, Richard C. Arnold and Ad Harris at the US Marine Hospital, Staten Island, successfully treated four patients with primary syphilis. Later in 1984, Arnold wrote about his earlier work:

> "*Syphilis was once a dreaded and dreadful disease involving millions of US citizens. Before the introduction of penicillin, the heavy-metal cure often caused thousands of deaths each year. The morbidity and mortality of the disease itself was horrendous, involving all ages from the fetus to the elderly.*"

During the golden era of antibiotic discovery, new alternative drugs to penicillin, such as doxycycline, azithromycin and clindamycin, also emerged to treat this disease. But sadly the optimism that the new antibiotic age would lead to the eradication of bacterial diseases like syphilis has been premature. Syphilis is not a disease of the past. On a worldwide scale, congenital syphilis is still a condition that affects pregnancy, causing serious health problems and death to babies. Current estimates indicate that in 2012, there were approximately 18 million cases of syphilis, with 5.6 million new syphilis cases in women and men aged 15–49 years globally. In the UK, the numbers of infectious syphilis diagnoses are at the highest since the mid-1950s, and this pattern is repeated on a global scale. Worryingly, although penicillin and its derivatives still remain an effective cure, we already have strains of syphilis that are now resistant to the newer, alternative drug treatments, such as azithromycin and clindamycin. The 'Great Pox' is still with us and serves as a stark reminder that prevention is still better than the cure.

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Comment

The voyages of Zika virus

Derek Gatherer

The announcement in May this year from the World Health Organization, that the Zika virus outbreak that began in October 2015 in the Cape Verde Islands off the west coast of Africa was an American variant of Zika virus, confirmed that Zika has now circumnavigated the world.

Unlike the first human circumnavigators in the 16th century, who sailed the world from east to west, Zika travelled in the opposite direction, heading east out of Africa to South-east Asia, then across the Pacific Ocean, through the Americas and finally back across the Atlantic to Africa. Zika was discovered by accident in 1947 in macaque monkeys caged in Uganda's Zika forest as part of a yellow fever monitoring study. A relative of yellow fever in the genus *Flavivirus*, and spread in much the same way by mosquitoes of the genus *Aedes*, Zika simply joined the growing list of obscure tropical viruses of no clinical importance, registering barely a dozen mild cases of fever and rash in humans over the next 60 years.

Obscure no more

Then in 2007, the isolated Micronesian island of Yap became the location of the first epidemic in humans. Subsequent serological investigation showed that the majority of the population of a few thousand were infected, and that most of them reported no symptoms. Zika's potential for disseminated transmission was amply illustrated, but it wasn't until 2013 that the clinical implications of this became clearer. In that year, an even bigger outbreak occurred in Polynesia, infecting tens of thousands and adding an unwelcome new symptom to Zika's clinical description – Guillain-Barré syndrome, an auto-immune paralysis. Even then, there were no fatalities, and it wasn't until Zika completed the trans-Pacific leg of its journey, arriving in Brazil during or before the 2014 World Cup, a wave of foetal microcephaly cases trailing nine months in its wake, that the world finally realised the seriousness of this new pandemic, with the WHO declaring a Public Health Emergency of International Concern (PHEIC) on 1 February 2016.

Out of Africa

Why Zika should suddenly cause so much trouble after decades, perhaps centuries, of obscurity, remains a mystery. We have good grounds to believe that Zika originated in Africa, for the classic population genetic reason – which also applies to human immunodeficiency virus (HIV) and hepatitis C virus (HCV) – that the African strains are more genetically diverse than the non-African ones, indicating an accumulation of variation over a longer period of time. Diversity within the Asian and American strains is comparatively small and Bayesian phylogenetic analyses have dated Zika's emergence from Africa in the early- to mid-20th century. Zika's sporadic appearances in Africa have been in a belt from Uganda, through the Central African Republic to Nigeria and Senegal in the west, and its first isolation outside of Africa was in Malaysia in the 1960s. Just as earlier slave trade movements probably exported Zika's relative yellow fever from Africa across the Atlantic to the Americas, it is easy to imagine later colonial migrations, possibly administrative, commercial or military, carrying Zika from the British colonies in East Africa to those in South-east Asia. The fact that it didn't reach the Americas along with yellow fever in the 18th century suggests that Zika was rare in West Africa at that time, and indeed we have no up-to-date information on Zika's incidence in Africa now, except for a handful of serological studies conducted mostly before 1980 which suggest that between 6% and 60% of the African population are exposed to Zika at some point in their lives. In Asia, the corresponding figures are lower: less than 15% at all sites tested, again consistent with a relatively recent arrival from Africa.

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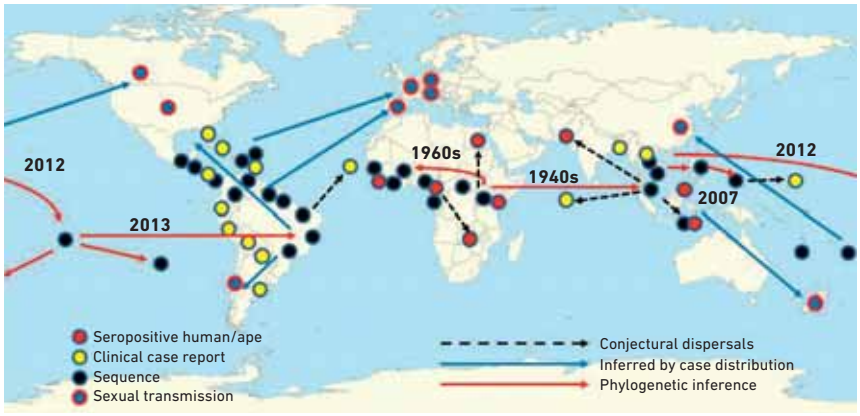
Increased virulence or lack of herd immunity?

We cannot currently reconstruct much concerning Zika's pre-history until we can obtain more genome sequences from both Africa and Asia, and Zika is so far proving a difficult virus to isolate and sequence. A short viraemia and odd compositional content mean that we simply aren't accumulating genomes as fast as we did with Ebola at the height of the West African outbreak in 2014–15. To a certain extent, however, quality is better than quantity. Most of the Brazilian and other Latin American Zika genomes are very similar, and the really interesting differences are likely to occur away from the leaves of the phylogenetic tree and down in the branches where Asian Zika diversified before setting off across the Pacific. Here is where the crucial genetic differences will be found if it is the case that Pacific/American Zika's apparently novel properties – wider range of symptoms, possibly a greater transmissibility, Guillain-Barré and microcephaly associations – are due to evolution of the virus. On the other hand, it is perfectly possible that strain differences are inconsequential genetic drift and that what we are really seeing in the Americas is simply yet another example of an introduced pathogen wreaking havoc in a population with no previous exposure. The Native Americans of colonial times suffered terribly from the introduction of smallpox, influenza, yellow fever and even cold viruses from the Old World, and Zika may simply be doing what many viruses tend to do when they enter a host population with no herd immunity.

What now for Africa?

The answer to this question will also have consequences for Africa. The Zika outbreak in the Cape Verde Islands is confirmed by the WHO as being of Brazilian origin, and has been associated with microcephaly. If the islanders have never been exposed to Zika before, we might regard this as a similar situation to Brazil. On the other hand, if African Zika has circulated in Cape Verde, we must wonder why it has not produced a cross-protective effect against the American strain. Meanwhile, Guinea-Bissau has just become the first mainland African country to report an outbreak in recent times. The strain involved is of African origin and not, unlike the strain in Cape Verde, an import from Brazil. What happens there may forewarn us of what might happen elsewhere in Africa. The crucial question is – can African Zika also cause microcephaly? We may soon have an indication from the macaque monkey model of Zika infection used in the vaccine development programme. The current experimental Zika vaccine is based on American strains (Brazilian and Puerto Rican), so if it protects against infection with an African strain, the converse will probably apply and we are unlikely to see a similar epidemic of microcephaly in Africa as we have recently seen across tropical Latin America.



Zika circumnavigates the world. The pattern of spread has been deduced from phylogenetics (red arrows), epidemiology (blue arrows) or by informed guesswork (dotted arrows). Underlying map, Vardion, Wikimedia Commons; arrows, circles and dates added by author

Zika Down Under

Meanwhile, Zika continues to be detected more sporadically in South-east Asia. The lower epidemiological intensity and absence, so far, of microcephaly complications suggests that South-east Asia, perhaps like Africa, has the herd immunity that the Pacific and Americas lack. Since the climate of Indonesia, and also some *Aedes* mosquito species, is shared by the tropical northern Australian coast, Zika might have been expected to have already arrived in Australia. However, what is different is the absence of a wild monkey species in Australia, which may deprive Zika of the animal reservoir it needs to maintain itself in an area where the human population is mostly immune. In Africa, red-tailed monkeys have been shown to be a Zika reservoir, and in Brazil, Zika has already been detected in marmosets and capuchins. Of course, Australia, like all countries, could also see Zika spread by sexual transmission. This is climate- and mosquito-independent, but we still have no idea if it is sustainable.

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Read more about Zika in the article *Pregnancy, the placenta and Zika virus infection* by Bill Rawlinson on page 00 of *Microbiology Australia* included at the back of this issue.

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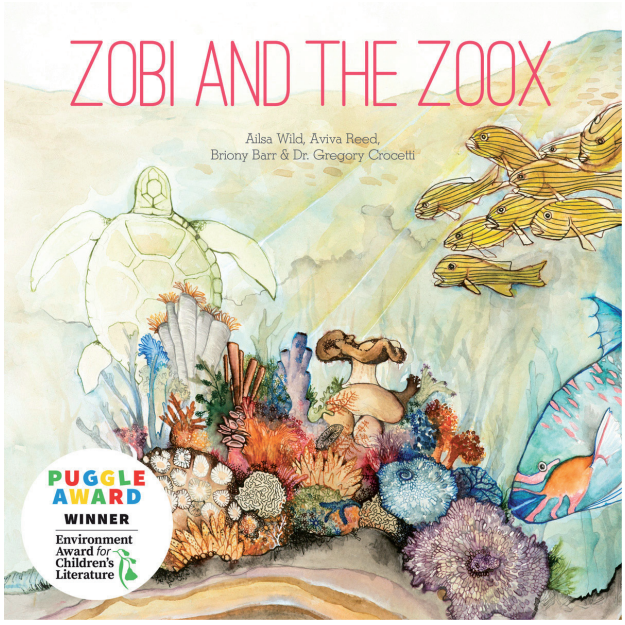

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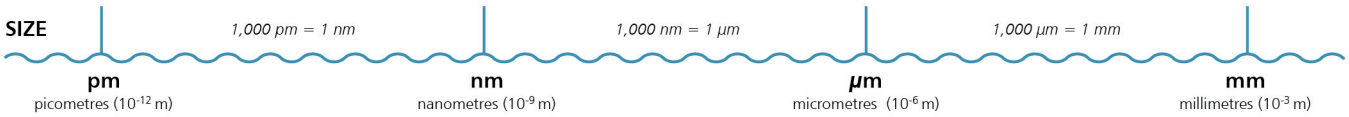

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