Travel – a risk factor for disease and spread of antibiotic resistance

Martin Angelin
In memory of my mother
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### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AmpC</td>
<td>Nomenclature from a study on penicillinas in the 1960s that still is in use, amp for ampicillin.</td>
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<tr>
<td>AUDIT-C</td>
<td>The Alcohol Use Disorders Identification Test – Consumption</td>
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<td>BWA</td>
<td>Burrows-Wheeler Aligner</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
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<tr>
<td>CMY</td>
<td>Cephamycin</td>
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<tr>
<td>CPE</td>
<td>Carbapenemase-Producing <em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>CTX-M</td>
<td>Cefotaximase - Munich</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>ESBL-PE</td>
<td>Extended-Spectrum Beta-lactamase Producing <em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>EUCAST</td>
<td>The European Committee on Antimicrobial Susceptibility Testing</td>
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<tr>
<td>FDR</td>
<td>Benjamini-Hochberg False Discovery Rate</td>
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<td>HCS</td>
<td>Healthcare student</td>
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<tr>
<td>HGT</td>
<td>Horizontal Gene Transfer</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
</tr>
<tr>
<td>IMP</td>
<td>Imipenemase</td>
</tr>
<tr>
<td>ISCR</td>
<td>Insertion Sequence Common Region</td>
</tr>
<tr>
<td>ISTM</td>
<td>International Society of Travel Medicine</td>
</tr>
<tr>
<td>KPC</td>
<td><em>Klebsiella pneumoniae</em> carbapenemase</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix-Assisted Laser Desorption Ionization – Time of Flight</td>
</tr>
<tr>
<td>MBL</td>
<td>Metallo-beta-lactamase</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal Inhibitory Concentration</td>
</tr>
<tr>
<td>NDM-1</td>
<td>New Delhi Metallo-beta-lactamase 1</td>
</tr>
<tr>
<td>NGS</td>
<td>Next Generation Sequencing</td>
</tr>
<tr>
<td>NHCS</td>
<td>Non-healthcare student</td>
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<tr>
<td>OXA</td>
<td>Oxacillin-hydrolysing</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PFGE</td>
<td>Pulsed Field Gel Electrophoresis</td>
</tr>
<tr>
<td>RA</td>
<td>Relative Abundance</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SARS</td>
<td>Severe Acute Respiratory Syndrome</td>
</tr>
<tr>
<td>SHV</td>
<td>Sulphhydryl variable (early assumption that a particular inhibition of SHV activity was substrate variable)</td>
</tr>
<tr>
<td>STD</td>
<td>Sexually Transmitted Disease</td>
</tr>
<tr>
<td>TD</td>
<td>Travellers diarrhoea</td>
</tr>
<tr>
<td>TDB</td>
<td>Travel and Tourist Database</td>
</tr>
<tr>
<td>TEM</td>
<td>Named after a Greek patient named Temoneira</td>
</tr>
<tr>
<td>VFR</td>
<td>Visiting Friends and Relatives</td>
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<tr>
<td>VIM</td>
<td>Verona Integron–encoded</td>
</tr>
<tr>
<td>VPD</td>
<td>Vaccine Preventable Disease</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
Abstract

As international travel is rapidly increasing, more people are being exposed to potentially more antibiotic resistant bacteria, a changed infectious disease epidemiology, and an increased risk of accidents and crime. Research-based advice is needed to adequately inform travellers about these risks. We studied travellers who sought advice from the Travel Medicine Clinic at the Department of Infectious Diseases, Umeå University Hospital, as well as university students from Umeå, Stockholm, and Gothenburg travelling abroad for study, research, and clinical exchange programs.

From retrospective data at the Travel Medicine Clinic, we found that pre-existing health problems were rare among travellers from Umeå seeking pre-travel health advice and vaccinations. In addition, we found that the travel destination and the sex of the traveller affected vaccination levels. Although hepatitis A is endemic to both Thailand and Turkey, compared to travellers to Thailand few travellers to Turkey visited the clinic for hepatitis A vaccination. The data also revealed that more women than men were vaccinated against Japanese encephalitis despite comparable trips.

A prospective survey study showed that travellers felt that the pre-travel health advice they received was helpful. Two-thirds of the travellers followed the advice given although they still fell ill to the same extent as those who were not compliant with the advice. Factors outside the control of travellers likely affect the travel-related morbidity. Compared to older travellers, younger travellers were less compliant with advice, fell ill to a greater extent, and took greater risks during travel.

In a prospective survey study, we found that healthcare students had higher illness rates and risk exposure when abroad compared to students from other disciplines. This difference was mainly due to the fact that healthcare students more often travelled to developing regions during their study period abroad. When abroad, half of all students increased their alcohol consumption and this was linked to an increased risk of theft and higher likelihood of meeting a new sex partner.

The healthcare students participating in the survey study also submitted stool samples before and after travel. These samples were tested for the presence of antibiotic resistance, both by selective culturing for ESBL-PE (Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae) as well as by metagenomic sequencing. About one-third (35%) of the students became colonised by ESBL-PE following their study abroad. The strongest
risk factor for colonisation was travel destination; for example, 70% of students who had travelled to India became colonised. Antibiotic treatment during travel was also a significant risk factor for colonisation.

The stool samples from a subset of study subjects were analysed using metagenomic sequencing. From this we learned that although the majority of resistance genes in the gut microbiome remained unchanged following travel, several clinically important resistance genes increased, most prominently genes encoding resistance to sulphonamide, trimethoprim, and beta-lactams. Overall, taxonomic changes associated with travel were small but the proportion of Proteobacteria, which includes several clinically important bacteria (e.g., Enterobacteriaceae), increased in a majority of the study subjects.

Clearly, there are risks associated with international travel and these risks include outside factors as well as the personal behaviour of travellers. We believe our results can be used to develop better pre-travel advice for tourists as well as university students studying abroad resulting in safer travel.
Summary in Swedish


Den ökande antibiotikaresistensen hos sjukdomsorsakande bakterier är ett av de största hoten mot hälsan idag och i framtiden. Resistenta bakterier sprids över världen och på senare år har resenärers roll i spridandet av antibiotikaresistens uppmärksammats. Det har visats att många resenärer bär med sig antibiotikaresistenta bakterier i tarmen även efter en kortare turistresa. Mekanismerna och konsekvenserna av detta är inte klargjorda och behöver undersökas vidare.

I detta avhandlingsarbete har vi undersökt resenärer som inför en resa besökt resevaccinationsmottagningen på infektionskliniken vid Norrlands universitetssjukhus i Umeå samt universitetsstudenter från Umeå, Stockholm och Göteborg som genomförde en del av sina studier utomlands. Vi har fokuserat på risker och rådgivning vid utlandsresa och i den senare delen av avhandlingsarbetet riktat in oss på risken för bärarskap med antibiotikaresistenta bakterier i samband med resa.

Vår första studie baserades på hälsodeklarationer från besökare till resevaccinationsmottagningen i Umeå samt på antalet givna vaccindoser under samma period. Resultaten visade bland annat att fler kvinnor än män erhöll vaccin mot Japansk hjärnhinneinflammation trots motsvarande
resmål, typ av resa och reslängd. Orsaken till detta är oklar och behöver studeras vidare. Detta fynd visar att resenärers beslut att acceptera ett föreslaget vaccin kan bero på fler saker än de medicinska rekommendationer de får.

Den andra studien i avhandlingsarbetet var en enkätstudie på besökare till resevaccinationsmottagningen i Umeå. De flesta av besökarna var nöjda med reseråden de fick på mottagningen men analysen av enkätsvaren visade att råden inte skyddade resenärerna mot sjukdom under resan. Flera orsaker kan ligga bakom detta fynd. Till exempel kan dåliga hygienrutiner på restauranger på resmålet vara orsaken till insjuknande i resediarré, den vanligaste sjukdomen hos resenärerna i studien. Detta är något som resenären själv har svårt att påverka. Våra resultat visar att en kritisk genomgång av de vanliga reseråden behövs för att värdera deras effektivitet.

Studien visade också att en högre andel av de yngre resenärerna blev sjuka jämfört med de äldre. De yngre resenärerna utsatte sig även för mer risker under resan, till exempel var det fler som inte tog sin malariaförebyggande medicin som ordinerat. Malaria är den infektionssjukdom som orsakar flest dödsfall bland resenärer och den är därför mycket viktig att förebygga.

Vår enkätstudie på utresande universitetsstudenter från Umeå, Stockholm och Göteborg visade att studenter som studerade utomlands hade högre sjukdomstal än turister. Hälsostudenter (bland annat medicinstudenter och sjuksköterskestudenter) hade högre andel hälsoproblem och hade ett mer uttalat riskbeteende (fler träffade en ny sexualpartner och fler tog större risker i trafiken) än studenter från andra utbildningar, detta trots att en högre andel av hälsostudenterna fick reseråd före resan. Hälften av studenterna ökade sin alkoholkonsumtion under resan och hög konsumtion var bland annat relaterad till ökad risk för att bli bestulen.

Hälsostudenter som deltog i enkätstudien för utresande studenter lämnade även in avföringsprov för detektion av antibiotikaresistenta bakterier, så kallade ESBL bakterier. ESBL bakterier i tarmen ger inga sjukdomssymptom, men bakterierna kan orsaka till exempel urinvägsinfektion och blodförgiftning. De typer av antibiotika som normalt används vid dessa infektioner är inte verksamma mot bakterier som har resistens orsakad av ESBL. En tredjedel av studenterna bar på dessa bakterier i tarmen efter hemkomst. Resmål var den största riskfaktorn för att bli bärare av ESBL bakterier, till exempel hade resenärer till Indien förhöjd risk. Även de som behandlades med antibiotika under resan hade högre risk för att bli bärare. Man bör därför undvika antibiotikabehandling under en utlandsresa om det inte verkligen behövs. Att arbeta inom sjukvården
utomlands ledde inte till ökad risk för bärarskap, något som inte har undersökts tidigare. Konsekvenserna av bärarskap av ESBL bakterier är osäkra för den enskilde men bidrar till ökningen av resistenta bakterier i Sverige och mer mottagliga individer kan bli sjuka av dessa bakterier. Infektioner med ESBL bakterier är svårare att behandla och kan leda till längre och svårare sjukdom samt att man behöver använda antibiotikasorter som dyrare och har mer biverkningar.


Original papers

This thesis is based on the following papers, which will be referred to in the text by the corresponding numerals.


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Introduction

Travel is an integral part of human society and has evolved as human society has evolved. We travel for many reasons - to escape violence and persecution, to find employment, to conduct business, or simply to engage in leisure activities and relaxation. In 2013, international tourist arrivals reached 1,123 million, or 36 every second [1]. University students are frequent travellers both as tourists and through international exchange programs. Students travel abroad not only to study but also to conduct research projects and to gain work experience. During 2012, 4.5 million students were enrolled in higher education outside their home countries [2].

Travel exposes people to many risks, including risk of diseases, risk of accidents, and risk of being the victim of crime. The magnitude of the risk depends on factors such as the travel destination, type of travel, and the risk behaviour of the traveller. Prevention is the key to reducing travel risks. Preventive measures include supplying travel vaccinations, prescribing malaria chemoprophylaxis, and providing relevant travel advice. The discipline of travel medicine has evolved to meet this need studying the epidemiology of travel related risks and developing relevant preventive strategies to help travellers stay safe and healthy.

To reduce travel-related risks, travellers should seek health-related pre-travel consultation. Survey studies performed at departure terminals in Europe, United States and Australia investigating travellers to developing countries have found that between 35% and 66% of travellers sought pre-travel health information [3-8]. Pre-travel health information was mainly acquired from primary healthcare providers; between 4% and 26% had visited a designated travel medicine clinic. Women were more likely than men to seek pre-travel health information [9]. A common reason for not seeking pre-travel health advice was that the traveller felt that he or she already possessed the relevant information [5, 7].

Travellers not only risk falling ill but also risk spreading contagious diseases when returning home [10]. Outbreaks of measles have often been linked to primary cases in travellers [10-12]. Other examples include the outbreak of smallpox in Stockholm, Sweden in 1963 [13], the outbreak of Chikungunya in northern Italy in 2007 [14, 15], the SARS pandemic in 2002-2003 [16], and the current Ebola outbreak in West Africa. In addition, travellers can spread antibiotic resistant bacteria; several recent studies have demonstrated that international travel involves a considerable risk of becoming colonized by antibiotic resistant bacteria [17-26].
Accidents in travellers

Accidents and cardio-vascular disease are the leading causes of death in travellers [27-34]. Although airplane crashes are among the most dramatic travel accidents, airplane crashes account for very few fatalities. In 2014, the Aviation Safety Network reported 990 deaths due to airplane crashes, an accident rate of one fatal passenger flight per 4,125,000 flights [35]. Every year many more travellers die in traffic accidents or drown. Injury death has been reported to be more common when abroad [28, 31] as well as more common in tourists than in the local population [28]. Young men have an increased risk of injury-related death abroad [28, 31, 36]. Incidence of death due to accidents has been difficult to assess due to the lack of reliable denominator data, but such data are available in a 2010 Finnish study by Lunetta et al. [31]. Lunetta et al. estimated mortality risk from land traffic accidents abroad to be 20.7/100,000 person-years. The risk for death in a traveller from all causes has been estimated to be 1/100,000 travellers per month of travel [37]. There seems to be a relationship between travel destination and number of accidental deaths. Although the results regarding which destinations have higher risk is inconclusive, it seems that an increased risk of accidental death during travel abroad exists on all continents [28, 31, 36].

Non-fatal injuries are common in tourists and are often the reason for travellers to seek medical assistance when abroad [28]. In a 2015 Finnish study, Siikamäki et al. used data on travellers requiring help of an assistance organisation for health problems and nationwide travel data, to calculate the incidence of illness and injury during travel [38]. The incidence rate of injury was 1.3-14.0 per 100,000 travel-days, depending on the region visited. Travellers to Southern Europe/Eastern Mediterranean region had the highest risk of injury.

Few studies have examined drunk driving as a risk factor for tourists [28], although some studies have reported high alcohol consumption in travellers [39, 40]. A study from the island of Crete in Greece found that alcohol use more often was the cause of traffic accidents with tourists than with locals [41].

Illness in travellers

Cardio-vascular disease is the most common cause of death due to disease in international travellers. Death from an infectious disease is much more rare, representing 1-2% of causes of death in international travellers. Most deaths due to an infectious disease are caused by malaria [28, 31, 37]. Although not
a common cause of mortality in travellers, infectious diseases are a major cause of travel-related morbidity. Illness rates in travellers are based on statistics related to notifiable diseases, retrospective and prospective survey studies, claims to insurance companies, and reports from international assistance organisations. In addition, illness incidence rates are based on incidence data from ill travellers who seek a healthcare provider after travel. True incidence rates are difficult to generate with these methods since reliable denominator data are not available. Prospective survey studies can generate illness data but are most often biased as they usually only analyse visitors to travel medicine clinics, a selective sample of travellers. Survey studies (foremost retrospective studies) also have an inherent problem, recall bias.

In their nationwide incidence rate study for illnesses and injuries in Finnish travellers, Siikamäki et al found that infectious diseases accounted for 60% of cases reported to the national assistance organisation [38]. Gastroenteritis (23% of all cases) and respiratory tract infection (21% of all cases) were the most common infectious diseases reported. The highest incidence of gastroenteritis was found in travellers to Africa (incidence 77/100,000 travel-days) and the highest incidence of respiratory tract infection was found in travellers to Southern Europe/Eastern Mediterranean (incidence 21/100,000 travel-days). The true incidence rates were probably somewhat higher than found in this study since milder illness during travel was not reported to the assistance organisation.

Surveys of travellers from Europe, North America, and Israel have shown that between 10% and 87% of travellers experience a health problem during travel [42-53]. This very wide range is explained by large differences in study sizes and different groups of travellers studied. Pooled data from these studies show that 47% (n=11,191) of travellers experience health problems while travelling. Risk factors for health problems during travel were travel length, travel destination, type of travel, and the age of the traveller (higher illness rates in younger travellers). Destinations on the Indian peninsula were associated with a higher risk of illness compared to other destinations. Most illnesses contracted during travel did not require hospitalisation abroad (<1%) [37, 45].

These survey studies found that the most common health problem for travellers was travellers’ diarrhoea, affecting between 9% and 46% of travellers, with a pooled illness rate of 32% (n=8969) [42, 44-48, 50-53]. Respiratory tract infection was the second most common health problem with an illness rate of between 5% and 26% and a pooled rate of 14% (n=8556) [42, 44-48, 50-52]. Dermatological problems were reported by
between 2% and 8% [42, 45, 48, 51] and fever of unknown origin by between 3% and 11% [44, 45, 48, 51]. Confirmed malaria cases were recorded in two studies with a pooled malaria rate of 0.6% (5/836) among travellers to malaria endemic regions. Four out of five cases reported compliance with chemoprophylaxis [45, 53]. These survey studies mainly investigated standard travellers and not long-term travellers or expatriates living in developing countries for extended periods (years). These latter groups of travellers are more likely to present with more serious illnesses [54] as exemplified by a survey study on volunteers stationed in developing countries [55]. More than half (54%) of volunteers in the group studied were on rotations exceeding 12 months. In this study, 11% were diagnosed with smear positive malaria. Unfortunately, compliance with chemoprophylaxis in the volunteers with malaria was not reported.

A few studies have investigated health problems in students studying abroad (mostly medical students) and have found a pooled incidence of 40% (n=1063) [56-58].

Several infectious diseases associated with travel were not identified in the survey studies due to their low incidence in travellers or the need for laboratory or clinical confirmation. Incidences of vaccine preventable diseases are discussed in another section below. Dengue is estimated to account for 2% of all illness in travellers returning from dengue-endemic regions. Travellers diagnosed with dengue have most commonly travelled to South-East Asia. In this region, dengue is now a more frequent cause of febrile illness than malaria for travellers [59].

Travellers are also at risk for sexually transmitted infections. One in five meet a new sex partner during travel and only around 50% use a condom when engaging in casual sex while travelling [60]. Legionella, leishmaniasis, schistosomiasis, mellioidosis, and leptospirosis are just some of the more exotic infections that can affect travellers. Their incidence in travellers is not known and information is mostly based on case reports such as data from the GeoSentinel network. The GeoSentinel network, established in 1995 by the International Society of Travel Medicine (ISTM) and the Centers for Disease Control and Prevention (CDC) [61], as of February 2015 consists of 58 collaborating travel/tropical medicine clinics around the world [62]. Each centre submits standardised reports on all cases involving travel-related illness to a central database in the United States. In February 2015, this database contained more than 230,000 individual reports. From these reports proportionate morbidity per 1000 ill travellers can be calculated. One study based on GeoSentinel data showed a clear sex related difference in morbidity during travel [63]. Men were more likely than women to have a
vector borne infection such as malaria and dengue fever and to have a sexually transmitted disease as well as viral hepatitis. Women were more likely than men to have travellers’ diarrhoea, respiratory tract infection, and urinary tract infections. Reasons for these differences are probably multifactorial, involving both biological and behavioural differences (e.g., risk taking).

Immigrants returning to their native country to visit – Visiting Friends and Relatives (VFR) – are also a risk group. Following travel, VFR travellers have a disproportional high number of cases of malaria, enteric fever (S. typhi and S. paratyphi), and hepatitis A [64-68]. Compared to other travellers, VFR travellers seek pre-travel health advice to a lesser extent and when they do, it is usually closer to departure [66, 69, 70]. In addition, VFR travellers are more likely than other travellers to decline a recommended vaccine [69].

**Travellers’ diarrhoea**

Travellers’ diarrhoea (TD) is defined as the passing of three or more unformed stools within 24 hours together with one additional symptom (abdominal cramps, tenesmus, nausea, vomiting, fever, or faecal urgency). Left untreated, TD usually lasts for four or five days with a short period of incapacitation. TD is most often caused by bacterial pathogens, but viral and parasitical pathogens also cause disease in travellers. The most important causes of TD are ETEC (Enterotoxic *Escherichia coli*) followed in decreasing incidence by EAEC (Enteroaggregative *Escherichia coli*), viruses (noroviruses and rotaviruses), *Salmonella*, *Campylobacter*, and *Shigella* [71]. Among parasitic infections, *Giardia lamblia* and *Cryptosporidium spp.* are most common [72]. The geographical variation in prevalence of different pathogens is considerable. In South-East Asia, *Campylobacter* is the most common pathogen, not ETEC [71]. Parasitic pathogens are a relatively more common cause of TD in Asia (especially South Asia) compared to other destinations [73].

Available ways to potentially prevent TD are many, including hygienic precautions, antibiotic prophylaxis, and the use of oral cholera vaccine. Hygienic precautions have repeatedly been shown to have little effect on the incidence of TD [48, 52, 67, 71, 72, 74-77]. This is likely explained by suboptimal hygienic standards in restaurants at travel destinations. Oral cholera vaccine has cross-protection against heat-labile ETEC. The effect on all causes of TD, however, is low and its use for this indication is not recommended [78, 79]. Three types of antibiotics are mainly used in the prevention and treatment of TD - ciprofloxacin, azithromycin, and rifaximin. Rifaximin, a broad-spectrum antibiotic with almost no systemic distribution
due to its low absorbance from the gut, has little effect on invasive TD caused by *Campylobacter, Salmonella*, and *Shigella* limiting its usage. Therefore, rifaximin is not registered in Sweden for the treatment of TD. Systemic antibiotics such as ciprofloxacin and azithromycin are highly effective in preventing TD, but they come with side effects and the risk of increasing antibiotic resistance, the latter is also the case with rifaximin [71, 72, 80]. To supply all travellers with a standby antibiotic regimen for self-treatment of TD is considered as standard practice by some [81]. Short course antibiotic treatment is highly efficient against unspecified TD [71], but treatment of TD with antibiotics has been linked to increased risk of gut colonisation with antibiotic resistant bacteria [24]. Since most cases of TD are mild with short periods of incapacitation, self-treatment should be reserved for patients with certain underlying medical conditions (e.g., type 1 diabetes or inflammatory bowel disease) that make them more vulnerable to the effects of TD.

**Vaccine preventable diseases**

Table 1 lists the diseases preventable by travel vaccination and the protection level normally achieved through vaccination. Incidence estimations of each disease are also listed. An individual assessment must be made for each traveller since the incidence rates of illness are highly affected by the type of traveller (e.g., pre-existing medical conditions and risk behaviour) and the type of travel. Due to lack of information, the quality of the data is not always optimal, although the data still provide an appreciation of the risk. Tetanus, diphtheria, polio, and measles are included in childhood vaccination programs in many countries, which affects incidence rates. Unvaccinated travellers have risks higher than indicated for these diseases.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Incidence in travellers (per month of travel if otherwise not stated)</th>
<th>Vaccine induced protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td>1/500,000 incidence per journey to Africa and Asia found in a study from the 1980s, current incidence most likely lower</td>
<td>High-moderate</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>Case reports</td>
<td>High</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>12.8/100,000 travellers</td>
<td>High</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>4.5-10.2/100,000 travellers</td>
<td>High</td>
</tr>
<tr>
<td>Influenza</td>
<td>1/100 travellers have febrile influenza-like illness</td>
<td>Moderate-low (seasonal vaccine)</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>1/300,000-1/1,000,000 incidence per journey</td>
<td>High</td>
</tr>
<tr>
<td>Measles</td>
<td>Case reports</td>
<td>High</td>
</tr>
<tr>
<td>Meningococcal disease</td>
<td>0.4/1,000,000 travellers, similar to levels in industrialized countries</td>
<td>High (conjugate vaccines)</td>
</tr>
<tr>
<td>Polio</td>
<td>Last case of imported Polio in industrialized countries was in 2007. Recent spread from Iraq and Syria to East Africa.</td>
<td>High</td>
</tr>
<tr>
<td>Rabies</td>
<td>1.1/100 travellers are bitten by dogs, 42 deaths/20yrs due to imported rabies in Europe, USA and Japan</td>
<td>High (post-exposition vaccination required)</td>
</tr>
<tr>
<td>Tetanus</td>
<td>Case reports</td>
<td>High</td>
</tr>
<tr>
<td>Tick born encephalitis</td>
<td>10/100,000 travellers to endemic regions</td>
<td>High</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1.28-2.8/1000 person-months of travel with PPD conversion, 0.06%-0.6%/1000 person-months of travel with active infection</td>
<td>Low in adults</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>17-33/100,000 travellers to South Asia, 1-2/100,000 to Africa, Middle East and South America, &lt;1/300,000 to Central America and the Caribbean</td>
<td>Moderate</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>10-50/100,000 travellers (West Africa and Amazonas region), based on epidemiological data on local populations, 10 confirmed cases/42 years in travellers</td>
<td>High</td>
</tr>
</tbody>
</table>

Table 1. Vaccine preventable diseases in travel medicine. The information in this table is mainly based on data from Steffen et al (2015) [82], but it is also based on data from Steffen et al. (2010) [83], Belderok et al. [84], and Morger et al. [85].
Vaccine preventable diseases (VPD) were rare among the diagnoses registered at GeoSentinel clinics between 1997 and 2007, only representing 1.5% of all cases [86]. The most common VPDs were enteric fever from *S. typhi* and *S. paratyphi* (48% of VPDs), acute viral hepatitis (hepatitis A: 26% of VPDs, hepatitis B: 9% of VPDs), and influenza (12% of VPDs). Lack of pre-travel advice was significantly associated with having a VPD as compared to other GeoSentinel diagnosis. The incidence rates of VPDs might seem low, but vaccination offers travellers important protection, as exemplified in a 2012-13 outbreak of hepatitis A in European travellers to Egypt. In this outbreak, 107 cases were reported and among the 43 cases who responded to a questionnaire, none had received hepatitis A vaccination before travelling to Egypt [87].

**Malaria prevention in travellers**

Malaria was the most common diagnosis among 6,957 patients with fever following travel and who received treatment at a GeoSentinel clinic between 1997 and 2006 [88]. Malaria is caused by protozoan parasites of the genus *Plasmodium*. Five species of *Plasmodium* can infect humans - *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*.*P. falciparum* is the principal cause of death due to malaria [89, 90]. Most cases of malaria in travellers are acquired in Sub-Saharan Africa [64-67, 91, 92], with the highest risk in West and Central Africa [92, 93]. Malaria is the infectious disease that causes the most deaths in travellers, and is therefore important to prevent [28, 31, 37].

Malaria in travellers is prevented by chemoprophylaxis and bite avoidance. The latter can be achieved by several methods; the most important is using an impregnated bednet. Other methods include the use of insecticides/repellents and wearing clothing that covers the arms and legs. Depending on the travel destination, bite avoidance and malaria information is sufficient malaria prophylaxis; however, if the malaria risk is more substantial, the use of chemoprophylaxis is recommended. In Sweden, healthcare providers are generally not encouraged to supply travellers with standby treatment for malaria [90].

Chemoprophylaxis prevention of malaria includes atovaquone/proguanil, chloroquine, doxycycline, and mefloquine [89, 90]. Due to widespread chloroquine resistance, its use is limited to malaria endemic regions of Central America. Resistance to mefloquine exists in parts of South-East Asia (mainly parts of Thailand, Cambodia and Burma). Each prophylactic regimen has side effects. If atovaquone/proguanil produces side effects, they are usually mild gastrointestinal distress and headache. With doxycycline,
the side effects are also usually mild and include gastrointestinal symptoms and local fungal infections. Doxycycline causes photosensitivity in approximately 3% of users, so prolonged sun exposure should be avoided. Chloroquine also has few side effects, mainly blurred vision, headache, and gastrointestinal symptoms [89, 90]. Mefloquine is also well tolerated among the majority of users, although insomnia, abnormal dreams, drowsiness, and gastrointestinal symptoms can occur. Very rare but serious neuropsychiatric adverse effects have been reported with mefloquine. These side effects include seizures, depression, and psychosis. Mefloquine should not be used in travellers with a history of psychiatric illness (including anxiety disorders and depression) and seizures [89, 90, 94].

**Travel risks and travel advice**

When assessing the risk with a specific disease in an individual traveller, many aspects need to be taken into consideration. These include the risk of contracting the disease, consequences of contracting the disease, available preventive methods, and side effects of these methods.

To determine the risk of a traveller contracting a certain disease, incidence rates in the local population at the travel destination can be used. Travellers however, are seldom exposed to the same risk for disease as the local population, limiting the usefulness of this information. Incidence rates in standard travellers exist for some diseases and this information is more helpful in assessing the risk for the individual traveller. As previously noted, these rates can be achieved through various methods requiring different methodological considerations and come with the risk of both over-reporting as well as under-reporting the true incidence [95]. Different exposures during travel also influence the individual risk. These include countries (and regions) visited, seasonal variations, living standard during travel, and travel in urban compared to rural areas. Individual factors also affect the risk for disease such as pre-existing medical conditions and risk behaviour. As mentioned, certain travellers (such as younger travellers and VFR travellers) have increased risk for illness during travel.

The individual risk for vaccine preventable diseases is weighed against the protection level of a vaccine as well as possible side effects of the vaccine. Most travel vaccines are well tolerated [82] but some come with the risk of more serious side effects. The tolerance for serious side effects from travel vaccines should be low. If there is an increased risk for side effects and an actual risk for disease, travellers should be advised to change their travel itinerary. This consideration is best exemplified by yellow fever vaccination. There is a very small risk for serious side effects, sometimes fatal, from
yellow fever vaccination – vaccine associated viscerotropic disease and neurotropic disease. This risk increases in people over 60 years of age and the administration of the vaccine after that age should be given only after careful consideration of the risks and benefits of vaccination [96].

Obviously, accidents, the greatest risk during travel, are not prevented by vaccination or prophylactic treatment, a fact that illustrates the importance of effectively communicating travel risks. Travellers rated the risk for accidents and STDs significantly lower than healthcare providers who advise and treat travellers [97]. Clearly, effective communication of travel-related risk is crucial. If travellers do not perceive the risks as important, the suggested preventive measures will probably not be acknowledged. It is important to identify pre-existing knowledge and beliefs, since conflicting information interferes with communication about risks [98]. Numerical data are the most straightforward way to show a travel-related risk, but these data can be difficult for a traveller to assess and are likely interpreted very differently by different travellers. Travellers’ interpretation of risk information and how to communicate risk information to travellers has received little attention in travel medicine [95, 98]. Despite all estimations on disease occurrence and attempts to communicate risk, other factors such as the price of the vaccine may have a stronger impact on a travellers’ decision to accept a recommended vaccine.

After identifying important risks of a specific trip and communicating these risks to a traveller, a healthcare provider needs to provide the tools a traveller can use to reduce these risks. For the majority of risks, the main tool is travel advice resulting in behavioural change. Because a great deal of travel advice exists, it is important to focus on the ones most important to the individual traveller. Full adherence to travel advice is unlikely, but through effective risk communication a traveller becomes more involved and understands the importance of the advice given, hopefully increasing their adherence to the advice.

General advice focuses on pre-existing medical conditions and their influence on travel preparation and travel as well as their management abroad. It is recommended that travellers be told about the importance of travel health insurance and be advised to learn more about the security situation of the travel destinations as well as check the availability of reliable healthcare, especially for more exotic destinations. Specific topics in pre-travel advice includes - accidents and injury, malaria and malaria prevention, food hygiene and management of travellers’ diarrhoea, sexual risk taking and STDs, dermatological problems in the tropics, water borne diseases (e.g., schistosomiasis), and the risk of rabies from contact with local
dogs [99]. Oral advice stressing the most important topics should be accompanied with written and/or web-based information for the traveller to study after the consultation.

**Antibiotics in clinical use**

Antibiotic treatment represents one of the most important medical achievements during the 20th century, as it has the potential to cure previously lethal diseases and enables several other medical achievements such as organ transplantation and aggressive chemotherapy through the possibility of treating concomitant infections associated with such procedures. The first antibiotics introduced in the treatment of bacterial infections were the sulphonamides in the mid 1930s and the following decades saw the introduction of several different classes of antibiotics (Table 2).
<table>
<thead>
<tr>
<th>Antibiotic class</th>
<th>Examples</th>
<th>When introduced</th>
<th>Target in bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphonamides</td>
<td>Sulfamethoxazole</td>
<td>mid 1930s</td>
<td>Folic acid synthesis</td>
</tr>
<tr>
<td>Beta-lactams</td>
<td>Penicillins, cephalosporins, carbapenems</td>
<td>1938</td>
<td>Cell wall synthesis</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gensumycin</td>
<td>1946</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Chloramphenicoles</td>
<td>Chloramphenicol</td>
<td>1948</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Erythromycin</td>
<td>1951</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Doxycycline</td>
<td>1952</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Rifamycins</td>
<td>Rifampicin</td>
<td>1958</td>
<td>RNA synthesis</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>Vancomycin</td>
<td>1958</td>
<td>Cell wall synthesis</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>Colistin</td>
<td>1959</td>
<td>Cell membrane</td>
</tr>
<tr>
<td>Pyrimidines</td>
<td>Trimethoprim</td>
<td>1962</td>
<td>Folic acid synthesis</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>Clindamycin</td>
<td>mid 1960s</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Ciprofloxacin</td>
<td>1968</td>
<td>DNA synthesis</td>
</tr>
<tr>
<td>Streptogramins</td>
<td>Synercid</td>
<td>1998 (discovered 1963)</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>Linezolid</td>
<td>2000 (discovered 1955)</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Lipopeptides</td>
<td>Daptomycin</td>
<td>2003 (discovered 1986)</td>
<td>Cell membrane</td>
</tr>
</tbody>
</table>

*Table 2. Antibiotic classes. Partly adapted from Davies et al. [100], Lewis et al. [101], Huovinen et al. [102] and Li et al. [103].*

Since the introduction of quinolones in the 1960s, no new classes of broad-spectrum antibiotics have been discovered. This is a major concern in light of the rapid increase in antibiotic resistance and it emphasises the need to use existing antibiotics in a responsible manner. A major problem in the development of new antibiotics has been to find compounds that can penetrate the bacterial cell wall. Other obstacles include toxicity issues with
candidate drugs and problems in the design of clinical trials. The potential earnings from development and sale of new antibiotics is less than with medication for chronic conditions, a fact that has decreased the interest from the pharmaceutical industry [101].

The evolution of antibiotic resistance

Antibiotic resistance genes were present in bacteria long before the introduction of the first antibiotics in clinical practice [100, 104, 105], indicating an adaptation to naturally occurring antibiotics [106]. A vast number and diversity of antibiotic resistance genes exists in the environment and some soil bacteria may even use antibiotics as their sole source of carbon [105, 107]. Ancestors to most clinically important resistance genes are found in both antibiotic producing and non-antibiotic producing bacteria in the environment [106]. Penicillinases, by which penicillin is hydrolysed and rendered inert, were identified in bacteria before the introduction of penicillin in the treatment of bacterial infections [100]. The selection pressure exerted by the widespread use and misuse of antibiotics since its introduction has resulted in the rapid increase in prevalence and complexity of antibiotic resistance.

Traditionally, it has been believed that the selection of antibiotic resistant strains from exposure to antibiotics occurs at antibiotic concentrations above the Minimal Inhibitory Concentration (MIC, the lowest concentration of an antibiotic that inhibits visible bacterial growth in vitro) of susceptible strains but below the MIC of resistant strains. However, it is now clear that antibiotic concentrations below the MIC of susceptible strains, so-called sub-MIC or sub-lethal concentrations, also infer a selection pressure favouring resistant bacterial strains [108]. Antibiotic concentrations at sub-MIC are common, existing in humans and animals receiving antibiotic treatment and in animals receiving antibiotics as growth promoters. Sub-MIC concentrations frequently occur in the environment as the result of human and animal excreta that contains antibiotics, from the agricultural use of antibiotics, and from industrial waste from factories producing antibiotics [108]. The mechanisms behind the selection pressure from sub-MIC concentrations of antibiotics are not clear, but the concept helps to explain the rapid global increase in antibiotic resistance.

Bacteria become resistant to antibiotics through different mechanisms, including - drug inactivation/modification (e.g., hydrolytic cleavage by beta-lactamases); alteration of the target site (e.g., the prevention of quinolones from blocking DNA gyrase and DNA topoisomerase IV); and reduced drug accumulation through reduced drug permeability and/or increased efflux.
(e.g., tetracycline efflux in gram negative bacteria) [101, 109, 110]. Because most resistance mutations result in an evolutionary disadvantage for bacteria in the absence of antibiotics, due to the resulting increase in biological fitness cost, it was assumed that without the selection pressure from antibiotics, the sensitive strains would out-compete the resistant strains. However, due to compensatory mutations, this initial fitness cost decreases, so restriction of antibiotic use will have limited effect on reducing the prevalence of resistant bacteria [111, 112].

Antibiotic resistance in bacteria can be innate or acquired. Acquired resistance may arise from chromosomal mutations or through the exchange of genetic information between bacteria. Horizontal gene transfer (HGT), first described in the 1940s, is one of the most important mechanisms for the transfer of antibiotic resistance genes between bacteria [113]. In HGT, genetic information is exchanged through one of three possible mechanisms: transformation - exogenous DNA transferred through the cell membrane; transduction - DNA transferred between cells via a phage (i.e., a virus); and conjugation – genetic material transferred by direct cell-to-cell contact [114] (Figure 1). In bacteria, especially in gram-negative bacteria, the most common form of HGT is conjugation via plasmids [100, 113].

Figure 1. Types of Horizontal Gene Transfer. Adapted from Furuya et al. [115] with the kind permission of the publisher.
Mobile genetic elements involved in the horizontal gene transfer of antibiotic resistance include plasmids, transposons, integrons, insertion sequences (IS), and insertion sequences common regions (ISCRs). Plasmids not only transfer resistance genes horizontally through conjugation but also vertically (to the next bacterial generation) through either incorporation in the host chromosome or through self-replication. Transposons are capable of transferring resistance genes to and from the chromosome, but they need to be in a plasmid to move between cells [116]. Integrons transport genetic material in the form of gene cassettes, which are small DNA molecules containing single genes, for example, resistance genes. Several gene cassettes can be inserted together in the same integron [117]. Intergrase is responsible for the excision and integration of DNA by integrons (called transposase in transposons). Integrons are an important part of HGT and although they may be transmitted independently, they are most often found on plasmids as well as form parts of transposons [118]. Traditionally, insertion sequences were regarded as simple transposons and were not seen as a means for transporting genetic material, but as a way to moderate the expression of genes. However, ISCR differs from other IS as they are capable of mobilizing adjacent DNA sequences; therefore they constitute a very mobile way of transferring resistance genes [119].

**Resistance in gram negative bacteria**

**Extended-spectrum beta-lactamases**

Beta-lactamases inactivates different groups of beta-lactam antibiotics depending on the type of beta-lactamase. In the 1970s, beta-lactamases TEM-1, TEM-2, and SHV-1 became important sources of resistance to broad-spectrum penicillins and were frequently found in *Enterobacteriaceae*, spread via plasmid-mediated horizontal gene transfer [120]. The SHV-1 enzyme was originally a chromosomal beta-lactamase in *Klebsiella pneumoniae*, but the origin of the TEM enzymes is still unclear. In the late 1970, as antibiotic resistance increased, several beta-lactam stable beta-lactam antibiotics were introduced. These new antibiotics included the oxyimino-cephalosporins that became widely used (third and fourth generation cephalosporins, mainly cefuroxime, cefotaxime, ceftriaxone, ceftazidime, and cefepime). Since the introduction of the oxyimino-cephalosporins, new TEM and SHV enzymes evolved with the capability of hydrolysing oxyimino-cephalosporins and were named extended-spectrum beta-lactamases (ESBL), a term introduced in 1988 [120, 121].

Other types of beta-lactamases include the OXA-group and the CTX-M group. The group of OXA-type beta-lactamases contains both enzymes that
hydrolyse the oxyminio-cephalosprins (i.e., are ESBLs) and beta-lactamases that do not. OXA-type resistance genes are traditionally found in *Pseudomonas aeruginosa*, but can exist in many other gram-negative bacteria [122].

**Cefotaximases – a success story**

Originally found in environmental *Kluyvera* strains, in 1989 the CTX-M genes were first reported in clinical samples in Germany (in Munich, hence the M in CTX-M) and South America [100, 123]. Initially found in *Escherichia coli, Klebsiella pneumoniae*, and *Salmonella* spp., CTX-M enzymes have also been detected (although not as frequently) in other *Enterobacteriaceae* and even in non-*Enterobacteriaceae* such as *Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Acinetobacter* spp., *Aeromonas* spp., and *Vibrio* spp. [123].

The first reports of infections caused by CTX-M producing bacteria came in the 1990s. After 2000, these beta-lactamases have become globally distributed in increasing prevalence. The CTX-M enzymes are now replacing the previously dominating TEM- and SHV-ESBLs in most parts of the world [113, 123-125]. CTX-Ms are not only hospital associated, as TEM-ESBLs and SHV-ESBLs mainly are, but also have spread in the community, mainly within the *Escherichia coli* species. This change could be one explanation of the rapid spread of CTX-Ms [122, 125, 126]. Resistance to additional antibiotics in bacteria carrying the CTX-M genes is common (e.g., resistance to quinolones and aminoglycosides), limiting treatment options even further as well as facilitating the dispersal of the CTX-M genes through co-selection processes [113, 123, 125, 127, 128].

The WHO compiles reports based on scientific and national data on levels of resistance in its six regions [129]. In the 2014 edition, individual reports collected from all regions described resistance levels of more than 50% to 3rd generation cephalosporins in *Escherichia coli* and *Klebsiella pneumoniae* [130].

**AmpC enzymes**

AmpC enzymes are most often chromosomally encoded beta-lactamases commonly (but not exclusively) found in *Enterobacteriaceae* and *Pseudomonaceae*. They also exist on plasmids (AmpC type CMY 2 being the most common), so they can appear in bacteria lacking the AmpC chromosomal gene, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* [113, 131].
Chromosomal AmpC expression is often low in Enterobacteriaceae but can be inducible by beta-lactam exposure (level of induction depending on the type of beta-lactam) and can be overexpressed due to mutation leading to resistance. Other mutations may render chromosomal AmpC-producing bacteria resistant to carbapenems through reduced influx (outer membrane porin loss) or enhanced efflux (efflux pump activation) of the antibiotic [131]. Plasmid mediated AmpCs are ESBLs and may in rare occasions be resistant to carbapenems in the presence of porin-deficiency. AmpC resistance genes often co-occur on the same plasmids with other beta-lactamase genes as well as genes encoding resistance to, for example, quinolones and aminoglycosides. Plasmid mediated AmpC resistance is not as common as other ESBLs, but it exists worldwide [131, 132].

**Carbapenemases**

With the increase in prevalence of ESBL-producing bacteria, the carbapenems provide an important treatment alternative. The emergence of enzymes that hydrolys carbapenems (first discovered in 1993) is therefore a considerable threat, severely limiting available treatment options. Bacteria with carbapenemase production often produce ESBL (with the exception of OXA-48 producers) and express other antibiotic resistance enzymes. Three classes of carbapenemases are of clinical importance - class A, B, and D [133, 134].

In class A carbapenemases, *Klebsiella pneumoniae* carbapenemases (KPCs) are the most common enzymes. Since the discovery of KPCs in the United States, it has spread throughout the world. Infections with KPC are mostly of nosocomial origin [133]. Class B metallo-beta-lactamases consist mainly of VIM and IMP types, which are endemic in, for example, Greece [113] (49% of *Pseudomonas aeruginosa* isolates in Greece were carbapenemase resistant in 2013 [135]). A recent addition to the group is the New Delhi metallo-beta-lactamase 1 (NDM-1). NDM-1 originated on the Indian peninsula and has now been reported throughout the world [136, 137]. It has been found in many species, but mainly in *Klebsiella pneumoniae* and *Escherichia coli* [133, 136], indicating potential of nosocomial and community dispersal. One of the most common enzymes in class D carbapenemases is the OXA-48 type carbapenemase. Many reports of hospital-acquired infections with this carbapenemase have originated in Turkey, but they have also been found in Europe and Africa. OXA-48-type enzymes are difficult to identify phenotypically and can be missed in a standard ESBL screening procedure. This difficulty highlights the need for improved screening methods for carbapenemases [133].
Definitions

With the increasing number and complexity of beta-lactamases, definition and categorisation becomes increasingly detailed. Two classification schemes are used – the molecular classification (i.e., the Ambler structural classification) and the Bush-Jacoby functional classification. The Ambler classification is based on amino acid sequence, dividing the beta-lactamases in classes A, B, C, and D. In the 2000 definition, the Bush-Jacoby functional classification scheme divides the beta-lactamases in sixteen groups (1, 1e, 2a, 2b, 2be, 2br, 2ber, 2c, 2ce, 2d, 2de, 2df, 2e, 2f, 3a, 3b), mainly based on which beta-lactam class they hydrolyse and if they are repressed by the beta-lactamase inhibitors clavulanic acid, sulbactam, and/or tazobactam [121, 138]. In these classifications the CTX-Ms, TEM-ESBLs, and SHV-ESBLs belong to molecular class A, functional group 2be.

In 2008, Giske et al proposed a more simplified definition intended to be more accessible to clinicians and non-scientists [121]. This definition is based on three main groups of beta-lactamases – the ESBL<sub>A</sub>, ESBL<sub>M</sub>, and ESBL<sub>CARBA</sub>. Beta-lactamases in the ESBL<sub>A</sub> group are inhibited by clavulanic acid and/or tazobactam and include CTX-M, TEM-ESBLs, and SHV-ESBLs. The ESBL<sub>M</sub> group consists of the plasmid mediated AmpC enzymes and the OXA-ESBL enzymes. All enzymes in the ESBL<sub>CARBA</sub> group have hydrolytic activity against carbapenems and are further divided in three groups A, B, (metallo-beta-lactamases) and D (OXA-carbapenemases).

The CTX-Ms have been divided, based on amino-acid sequence, into six groups or clusters, named after the first member discovered in each group – CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and CTX-M-45; sometimes the CTX-M-45 group is not listed as a separate group [124]. Currently 168 different CTX-M types are known [139] and CTX-M-14 and CTX-M-15 are the most common CTX-M types worldwide (belonging to the CTX-M-1 cluster) [140].

Metagenomics

Bacteria in the human gut outnumber human cells by 10 to 1 and the number of genes in the gut flora outnumber human genes by 100 to 1 [107]. The many different microbes in the gut constitute a microbial community, a microbiome. The majority of bacteria in the gut microbiome are not readily culturable [141, 142], although this can to some extent be due to insufficient anaerobic culturing methods [143]. The study of genes in the microbiome can be performed using PCR methodology. PCR targets the genes of interest using specific primers. This method makes it possible to find genes where
the primers fit perfectly with the nucleotide sequence while new variants of genes as well as novel genes will not be captured [107]. New techniques for massive parallel sequencing, Next Generation Sequencing (NGS), have made it possible to sequence the total DNA of microbiomes (e.g., the human gut microbiome) overcoming some of the limitations of PCR using specific primers. Such sequence data are called the metagenome and the sequence data analysis is labelled metagenomics [142].

Metagenomics allows for the study of the bacterial composition of the gut. The gut microbiome of one individual harbours at least 160 different prevalent bacterial species and in total more than 1000 different prevalent species have been detected in all individuals studied [144]. Firmicutes and Bacteroidetes are the dominating phyla in the microbiome of individuals examined and three enterotypes have been detected, characterised by the variation in levels of three genera – Bacteroides, Prevotella and Ruminococcus [145]. Although the classification into enterotypes may oversimplify the composition of the microbiome [146], it indicates the presence of a limited number of balanced microbial compositions across individuals. The gut microbiome composition is stable over time [147, 148] and different compositions have been linked to obesity as well as the development of disease (e.g., autoimmune diseases such as diabetes) [149].

Metagenomic sequencing can detect antibiotic resistance genes present in all gut bacteria in sufficient numbers. This includes resistance genes in bacteria culturable as well as bacteria non-culturable by standard laboratory methods. The collection of all resistance genes from an entire bacterial community like the human gut has been named the resistome. Metagenomic sequencing has indeed shown a vast number of previously undetected resistance genes in the gut microbiome [107, 150]. The clinical importance of these genes, many not linked with culturable, pathogenic bacteria, remains to be seen. Resistance genes in non-pathogenic bacteria in the gut may act as a resistance reservoir for more pathogenic strains transferring resistance genes through, for example, horizontal gene transfer.

**Faecal carriage of ESBLs**

Gram-negative bacteria causing clinical infections most often originate in the gut microbiome. Infections with CTX-M producing bacteria have been shown to be hospital-acquired as well as community-acquired [122, 125, 126]. Studying faecal carriage rates of ESBL-producing *Enterobacteriaceae* (ESBL-PE) may help explain the rapid increase in prevalence of these infections. Faecal colonisation of ESBL-PE in the community was first reported in Spain (2001) and Portugal (2002) [125]. From initial low
numbers in the early 2000s the rates have increased significantly towards the end of the decade and early 2010s. The geographical differences are apparent. In Europe, the levels are often between 5%-10% and in parts of South East Asia the levels are >60% [125]. Figure 2 shows the number of EBSL-colonised individuals in various regions of the world. The community carriage level in Sweden was found to be 4.8% in a 2012-2013 joint study by the Swedish Public Health Agency, National Food Agency, and the National Veterinary Institute [151].

![Figure 2](image)

**Figure 2.** Community faecal ESBL-PE carriage levels in 2010 in the six WHO regions [129]. Size of the bubble symbolises number of ESBL-PE carriers in the community. Stars represent countries with available data. Adapted from Woerther et al. [125] with the kind permission of the publisher.

**Risk factors for infection and colonisation with ESBL-PE**

Several risk factors for infection with ESBL-PE producing bacteria have been identified, including: previous hospital treatment, antibiotic use (specified as quinolones in some studies), nursing home residency, increasing age, co-morbidities (such as diabetes mellitus, haemodialysis, cancer, and heart disease), sex (both men and women have been shown to have higher risk), and international travel (travel to Asia has the highest risk) [127, 152-160].

Studies on risk factors for faecal colonisation with ESBL-PE have identified similar factors as in studies on infections with ESBL-PE. The most significant risk factors identified were previous hospitalisation and antibiotic use [161-165]. Other risk factors identified were previous international travel [166, 167], a family member with an ESBL-PE infection [168, 169], and living
with a pet [163]. One study found that higher education level was protective of colonisation [163].

**The role of international travel for the spread of antibiotic resistance**

Hospital treatment abroad has been identified as a significant risk factor for colonisation with various antibiotic resistant bacteria including ESBLs [170-172]. Treatment in an intensive care unit abroad infers an even higher risk of colonisation [172]. As a result of this high risk of colonisation, some European countries, such as Sweden and France, screen for antibiotic resistant bacteria in patients transferred from foreign hospitals [173].

As previously noted, travel has been identified as a risk factor for infection and colonisation with ESBL-PE. Colonisation levels of between 18% and 26% have been found in faecal samples of travellers with travellers’ diarrhoea [174-176]. Prospective studies have investigated travellers from countries with low carriage levels travelling to regions with high carriage levels. These studies show post-travel carriage levels of between 21% and 47% [17-21, 23-26]. Several travel-related risk factors for colonisation have been identified; travel destination [18, 20, 21, 23-26], travellers’ diarrhoea [18, 20, 24-26], antibiotic treatment during travel [24, 26], increasing age (highest risks seen in ages >65 years) [20], longer travel [22], VFR travellers [22], certain food items (e.g., ice cream and pastries) [22], and staying at all-inclusive resorts [26]. The travel destination is the most important risk factor and destinations with highest risk were found in South Asia and South East Asia followed by East Asia, the Middle East, and North Africa.

Plasmid-mediated AmpC enzymes were registered in two of the prospective studies on travellers, reporting a post-travel colonisation level of 6% and 9%, respectively [20, 26]. Colonisation with bacteria carrying carbapenemases have been reported in prospective studies, but at a very low frequency – 1/170 travellers (NDM-1, travel to India) in one study [22] and 3/574 travellers (1 NDM-1, 2 OXA-181, travel to India) in another study [26]. Several studies showed no carbapenemase colonisation [20, 21, 24, 25].

**Consequences of antibiotic resistance**

Colonisation with multidrug resistant bacteria has been shown to be associated with increased mortality as well as higher healthcare costs [170, 177]. Infections with ESBL-PE have been shown to increase mortality, to result in longer hospital stay, to delay correct treatment, and to generate higher healthcare costs [130, 160, 178, 179].
Today, national and international efforts are being made to reduce antibiotic resistance. For example, in March 2015 a national action plan to combat antibiotic-resistant bacteria was initiated in the United States [180] and in May 2015 the WHO introduced a global action plan on antimicrobial resistance and outlined five strategic objectives in order to achieve this goal [181]. These objectives include improving awareness, strengthening knowledge though surveillance and research, reducing incidence of infection, optimising use of antimicrobial agents, and investing in new medicines, diagnostic tools, and vaccines.

The following is the key message from the side-event on antibiotic resistance at the sixth World Health Assembly in May 2015 [182]:

*Antibiotic resistance is a rapidly evolving health issue extending far beyond the human health sector.*

*Awareness of the seriousness of the situation and the need for urgent action is required at the highest political level, globally and at country level.*
Aims

To investigate demographics, vaccinations received, and travel patterns among travellers from Umeå, Sweden including assessment of possible differences related to the sex and age of the travellers. \textit{(Paper I)}

To investigate if pre-travel health advice is relevant to travellers, if they comply with the advice, and if compliance is protective of travel-related illness. \textit{(Paper II)}

To investigate travel-related risks and risk behaviour in students studying abroad in order to provide research-based travel advice to this group. \textit{(Paper III)}

To investigate the faecal colonisation rate and risk factors for acquiring ESBL-PE and CPE among healthcare students taking pre-clinical and clinical courses abroad. \textit{(Paper IV)}

To investigate if international travel affects resistance gene abundance and the taxonomical composition in the gut microbiome with the use of explorative shotgun metagenomic sequencing. \textit{(Paper V)}
Material and methods

Material

Umeå, a city with a population of 119,000, houses the most northern university hospital in Sweden. The Travel Medicine Clinic at the Department of Infectious Diseases, Umeå University Hospital is part of the public healthcare sector in Sweden and administered by the County Council of Västerbotten. Visitors to the clinic pay a consultation fee (300 SEK, approximately 35 USD) and vaccinations are paid at cost price. During study I and II (i.e. between 2005 and 2011), the Travel Medicine Clinic averaged about 1500 doctors visits and 7050 nurses visits each year.

Paper I

In paper I, we analysed individual pre-consultation health declarations from visitors to the Travel Medicine Clinic in Umeå. The health declarations detailed sex, age, travel destination, and length of travel, as well as medical history, including chronic disease and potential immunosuppressive disorders or medication, relevant allergies, and pregnancy. Vaccination expenditure data were also collected. Between January 2005 and April 2008, 16,735 health declarations were collected at the Travel Medicine Clinic and from these every 10th declaration was consecutively chosen for analysis. To compare travel patterns among study subjects to travel patterns in the general population in Umeå, data from the Travel and Tourist Database (TDB) [183] were used. The TDB is a commercial on-going telephone survey that every month questions a random selection of the Swedish population regarding recent travel. The results are then statistically analysed to estimate travel patterns within the entire population.

Paper II

Visitors to the Travel Medicine Clinic in Umeå who were scheduled to see a doctor (embarking on trips for which multiple vaccines and/or malaria prophylaxis may be needed) between October 2009 and April 2012 were invited to participate in a prospective survey study. During their visit to the clinic, they received vaccinations, prescription of malaria prophylaxis, as well as oral and written travel health advice on hygiene and food security, travel diarrhoea, malaria, skin afflictions, animal bites, traffic accidents, drowning, sexually transmitted diseases, and recommendations on appropriate medications to bring during travel. At inclusion in the study, a short pre-travel survey with travel data was administered and approximately two
weeks following travel a second post-travel survey was administered. In total, 1059 study subjects completed both surveys and were included in the analysis.

**Paper III**

Swedish healthcare students (including medical students, nursing students, physiotherapists, dentists, dental hygienists, dieticians, speech therapists and psychologists) and non-healthcare students studying outside the Nordic countries between April 2010 and January 2014 were eligible for participation in this prospective survey study. Students from universities in Umeå, Stockholm, and Gothenburg were invited to participate. The international offices at the participating universities identified departing students. Before travel, a short pre-travel survey with travel data was administered and approximately two weeks following travel a post-travel survey was sent to the students. In total, 335 study subjects completed both surveys and were included in the analysis.

**Paper IV**

Healthcare students participating in study III were also invited to participate in a study investigating faecal colonisation with ESBL-PE before and after study-related travel abroad. Study subjects collected rectal swabs themselves at home, and sent them to the clinical microbiology laboratory in Umeå through regular mail. The swabs were screened for ESBL-producing and carbapenemase-producing bacteria, and antibiotic susceptibility testing was carried out on the ESBL-producing isolates. Since the subjects also participated in study III, survey data on travel destination and duration, antibiotic treatment during travel, and symptoms of diarrhoea were available and used for the analyses. In total, 99 study subjects submitted a full set of faecal samples.

**Paper V**

Students participating in study IV who travelled to the Indian peninsula or Central Africa were consecutively included in study V. To investigate travel-related effects on antibiotic resistance in the gut microbiome without the influence of antibiotic treatment, subjects who took antibiotics six months before travel or during travel were excluded. The study subjects collected their own faecal samples in sterile tubes and the tubes were sent via regular mail to the clinical microbiology laboratory in Umeå on the same day the samples were collected. At the laboratory, the samples were immediately frozen and stored at -20°C until analysis. As the subjects participated in
In total, 35 study subjects were included, 17 travelled to Central Africa and 18 to the Indian peninsula.

**Methods**

**Survey construction**

Questions on illness and compliance with malaria prophylaxis used in study II and III were previously validated in a study by Ahlm et al. [42]. In study II, questions on health advice, sexual behaviour during travel, and protective measures against accidents and crime were added. These questions were also used in the questionnaire in study III. In study III, questions on housing, healthcare contact, perceptions of the studies undertaken during the travel period, as well as questions on alcohol use (the AUDIT alcohol consumptions questions (AUDIT-C) [184-186]) were added. Both questionnaires were tested on a control group of ten individuals. These tests resulted in changes in some of the language. Most questions were quantitative with response alternatives provided. When open-ended questions were used, the results were analysed quantitatively. The questionnaires were available both in written form and as a web survey in the software Lime Survey [187]. The majority of study subjects used the web survey (79%).

Validity and reliability are important concepts in survey construction. Validity means that the survey measures what it is intended to measure without systematical errors and reliability means that repeated measurements give similar results without random errors [188]. In the construction of the surveys for study II and III, measures to ensure validity were the use of previously validated questions, testing on a control group, and by knowledge of the research area. Due to the nature of the survey questions, reliability testing with the test-retest method was not feasible. Furthermore, since the surveys contained no indexes, measurements of internal consistency were not needed.

**Detection and characterisation of ESBL-producing bacteria**

Screening for EBSL-PE was performed on chromogenic culture media. Positive isolates were analysed with culture-based methods according to EUCAST guidelines [189]. The definition of ESBL-producing Enterobacteriaceae by Gieske et al. [121] was used, and is currently the standard definition used in clinical laboratories throughout Europe. Antibiotic susceptibility testing (including cefotaxime, ceftazidime,
piperacillin/tazobactam, and meropenem) was done by disc diffusion. E-tests® (bioMérieux) were used to test for the presence of the ESBL phenotype (CTX-M, SHV, and TEM enzymes) [121].

To detect CPE that do not produce an ESBL (mainly OXA-48/OXA-181 producers), supplementary screening was performed [133, 190, 191]. Before screening on chromogenic media, all samples were tested by disc diffusion for susceptibility to ceftazidim, meropenem, and piperacillin/tazobactam. Reduced susceptibility to piperacillin/tazobactam prompted further analysis with susceptibility to temocillin. Isolates with reduced susceptibility to temocillin were tested with the CT-103 XL microarray (Check-Points Health B.V.).

Phenotypic species identification of ESBL positive Enterobacteriaceae isolates was done with the API® (API-20E, bioMérieux) identification system. Species identification of non-ESBL isolates as well as non-Enterobacteriaceae isolates growing on the chromogenic media was performed with MALDI-TOF-MS using a Bruker Daltonics Microflex LT mass spectrometer.

A few subjects in study IV were positive for ESBL both before and after travel. To see if the isolates post-travel were the same as pre-travel or acquired during travel, the isolates were analysed by pulsed field gel electrophoresis (PFGE) as previously described [192]. Visual inspection was used to identify PFGE patterns. PFGE uses restriction enzymes that cleave DNA at specific sites, generating DNA fragments of different lengths. These lengths distinguish different strains of bacteria. The DNA fragments are separated by size in an agarose gel with the help of an electrical field.

**DNA sequencing of faecal samples**

DNA was extracted from the faecal samples using the QIAamp® DNA Stool Mini Kit (QIAGEN) and following quality control for quantity, purity and shearing sent to the Science for Life Laboratories (SciLifeLab) in Stockholm, Sweden. At SciLifeLab, paired-end sequencing libraries (2x100 bp) were prepared using the TrueSeq DNA Kit. Sequencing was done using Illumina HiSeq2000 technology. DNA from the bacterial ESBL-producing strains identified in the culture screening was extracted using QIAamp® Mini Kit (QIAGEN). These samples were sequenced using Illumina MiSeq technology at FOI (Swedish Defence Research Agency), Umeå, Sweden.

Illumina technology uses next generation sequencing (NGS). Instead of individual reactions as with the traditional Sanger method, NGS sequencing
occurs in a massively parallel fashion in spatially separated arrays for clonally amplified DNA templates [193, 194]. The forward DNA strands in each cluster are sequenced by synthesis using fluorescence-labelled terminators. The Illumina platform was chosen due to the high output per sequence run and our research group’s familiarity with the method. We aimed for a very high sequence coverage (number of times each base pair is covered when aligning sequence reads) to also find less abundant resistance genes.

**Bioinformatic analysis of sequenced DNA**

All reads from the metagenomic samples and from the ESBL-P *Escherichia coli* isolates were quality filtered using Trim Galore! version 0.2.8 [195]. Reads from the *E. coli* isolates were assembled using SPAdes [196], and resistance genes were identified using the Resqu database version 1.1 [197].

Human DNA was removed from the metagenomic datasets by alignment to the hg19 human genome reference assembly (using Bowtie2 [198]). The metagenomic reads were scanned for bacterial 16S rRNA small subunit sequences using Metaxa2 [199] and the numbers of 16S rRNA sequences from each identified taxa were normalised to the total number of 16S rRNA sequences in that sample. Using Vmatch [200] with the Resqu database as a reference, we identified resistance genes in the metagenomic reads. To avoid overestimating the resistance gene counts, a strict definition of resistance genes was used by the choice of database (only containing resistance genes with a documented resistance phenotype) and only allowing for two mismatched amino acids between reads and the database reference (this strategy was used to account for sequencing errors and minor variability). After normalisation for gene length, the resistance gene abundances in each sample were also related to the total number of bacterial 16S rRNA sequences in that particular sample.

The reads from each metagenomic sample were *de novo* assembled using Ray Méta [201]. Open reading frames were identified with Prodigal [202] and mapped to the Resqu database. The metagenomic reads and the assembled contigs were mapped against the Human Microbiome Project gastrointestinal tract reference genome collection using BWA [203]. All reads were also mapped (using Bowtie2) to all the contigs in all samples to estimate their abundance in individual samples. Johan Bengtsson-Palme, first author of Paper V, performed the bioinformatic analysis.
Statistical methods

In Papers I-IV, the Chi-square test was used for categorical data, the Mann-Whitney U-test was used to compare continuous variables, and binary logistic regression was used for regression analysis. In the bioinformatic analysis in Paper V, paired Student's t-test was used on log10-transformed values to assess abundance changes in resistance genes and taxonomic groups. Correlations between resistance gene abundance and demographics as well as travel data were analysed using linear regression. Due to the nature of the data (i.e., the small number of individuals and many types of resistance genes and taxonomic families), correction for multiple testing employing a Benjamini-Hochberg False Discovery Rate (FDR) [204] was used. A p-value as well as an FDR of ≤0.05 was regarded as statistically significant.
Results and discussion

Paper I – A travel medicine clinic in northern Sweden

During the study period (2005-2008), the Travel Medicine Clinic in Umeå was the sole provider of travel vaccinations and travel health advice in the Umeå area and for some vaccinations (mainly Japanese encephalitis vaccine and Yellow fever vaccine) in Västerbotten County (population 262,000).

Surprisingly few of the visitors to the Travel Medicine Clinic in Umeå (6%) had medical conditions (allergies, immunosuppression, or other serious chronic diseases) that could have an impact on vaccination recommendations or recommendations on malaria prophylaxis. In other studies, levels between 11% and 26% have been found [205-208], although the definition of which medical conditions are included varies somewhat between studies. The reason for our lower level of travellers with chronic diseases is not known. Perhaps travellers from Umeå with more serious health conditions received travel advice and vaccinations from their treating physician.

In Paper I, we found that more women than men received vaccination against Japanese encephalitis for comparable trips (62% compared to 38%, \( p<0.05 \)) and women also spent more money than men on vaccinations. Data on sex differences in travel vaccination rates is scarce. Lu et al. found that vaccination levels of hepatitis A in travellers to endemic countries were higher in men than women [209]. In a 1996 survey study, McIntosh et al. showed that women reported more travel-anxieties than men [210]. The reason for our finding of different vaccination levels between men and women is not known, but highlights that the travel health consultation is a dialogue and the outcome is not only affected by medical recommendations.

Travel distance was found to affect vaccination levels. Seroprevalence of hepatitis A is comparable in Thailand and Turkey, so the risk to travellers for hepatitis A infection is fairly equal for the two countries [211]. Data from the Travel and Tourist Database [183] show that both destinations are as common among travellers from Umeå (7% and 6%, respectively). Despite the equal risk and equal number of travellers, Thailand was the travel destination of 32% of visitors to the Travel Medicine Clinic in Umeå compared to 6% going to Turkey. Hepatitis A vaccination alone is mainly recommended for both destinations. Travellers are apparently less likely to acknowledge health risks for shorter trips compared to more distant travel. German data also show that travellers to Turkey and other Mediterranean
countries with hepatitis A risk fail to get vaccinated [212]. All hepatitis A cases in Sweden are reported to the Public Health Agency. From 2004 through 2014, three times as many hepatitis A cases were reported to have been contracted in Turkey compared to Thailand [213]. It is important that travellers are made aware that there may be a need for travel vaccination even for shorter trips.

Paper II – Evaluating travel health advice

Most travellers (95%) found the travel health advice received at the Travel Medicine Clinic in Umeå was useful. Around half (40%) reported that the advice helped them avoid illness and/or accidents. Compliance with the advice was protective of illness during travel in the bivariate analysis but not in the multivariate analysis. Why compliance with advice was not protective of illness could be due to problems with the study design, poor recall among travellers of advice received [214], travel-related factors beyond the control of the traveller, and the appropriateness of the advice.

We only measured reported adherence to the travel advice and not actual adherence. The survey questions were also formulated in a more general fashion without asking for details about the level of compliance and its consistency throughout the trip. The study subjects were asked to list advice they felt was missing, but they were not asked which advice they received that was less relevant. Factors at the travel destination not easily influenced by the travellers probably had an impact on travel-related illness. These factors include hygienic standards at restaurants [74, 75, 215] and the local traffic situation (including lack of seatbelts). It is well documented that standard hygiene advice has limited effect on the incidence of travellers’ diarrhoea [48, 52, 67, 71, 72, 74-77]. As with hygiene advice, most travel-related advice is evidence based rather than research based, but attaining true research-based advice is difficult. Nevertheless, our results point to the need for a critical and comprehensive review of the current standard advice. Despite these problems, positive effects from travel advice can be seen, as in a recent study based on data from EuroTravNet (GeoSentinal network). In this study, recipients of pre-travel consultation had significantly lower proportionate morbidity of P. falciparum malaria, acute hepatitis, and HIV/AIDS compared to travellers who did not receive pre-travel advice [67].

Even if the level of reported compliance with malaria chemoprophylaxis found among travellers from Umeå (67%) was similar to other studies (47-89%) [51, 76, 216-219], compliance still needs to be improved. Malaria is the infectious disease that causes the most deaths of travellers [37] and is thus crucial to prevent. In fact, one of the most important risk factor for death due
to malaria in travellers is semi- or non-compliance with chemoprophylaxis [220]. Some travellers reported very low to no actual malaria risk at their destination as the reason for reduced adherence to chemoprophylaxis. Thus the level of compliance might actually be higher when there is an actual risk of malaria. It is difficult for a travel medicine practitioner to correctly estimate site and seasonal specific malaria risk for all destinations [37]. The level of compliance in travellers to Sub-Saharan Africa was higher than the average compliance level. This finding supports the idea that travellers are more likely to take their prophylaxis when the malaria risk is high. In both Paper II and III, forgetfulness was a common reason for reduced adherence to chemoprophylaxis. The traveller should be instructed to have a system with regular reminders for taking their chemoprophylaxis. In Paper II, side effects were a common reason for cessation of chemoprophylaxis. Informing travellers of possible side effects might affect the number of travellers that stop taking their malaria prophylaxis when side effects occur [98].

In Paper I, we could see that younger travellers tend to go on trips with longer duration than older travellers. Previous studies have shown that younger travellers have higher incidence of travel-related illness [42, 48, 50, 51, 71, 75, 221]. This study showed that younger travellers were a risk group in several aspects. They indeed had higher levels of travel-related illness, but were also more likely to be involved in traffic accidents and being the victims of crime (theft/robbery) as well as taking greater risks during travel (e.g., renting motorcycles and meeting new sex partners). Compared to older travellers, younger travellers reported lower adherence with travel advice, including adherence to malaria chemoprophylaxis, and they reported that the travel health advice they received was less relevant to their travel. This last fact is an important finding and needs to be addressed in order to reduce travel-related risks in this group. Focus group discussions targeting younger travellers could yield valuable insight. New ways of relaying the information should also be investigated, for example, through smart phone applications and social media.

Paper III – Illness and risks when studying abroad

More students had health problems during travel compared to tourists examined in Paper II (52% compared to 40%). The levels of health problems found in Paper II and III corresponds to data from previous studies. Pooled incidence estimates from survey studies of health problems in tourists and study abroad students are 47% and 40%, respectively (see “Illness in travellers” section in the introduction).
Healthcare students (HCS) were more likely than non-healthcare students (NHCS) to suffer from travellers’ diarrhoea and to travel to a malaria endemic country. In addition, HCS were less likely to practise safe sex than NHCS. More HCS were involved in a traffic accident and HCS were more likely to rent a motorcycle. Why HCS, despite receiving more pre-travel health information compared to NHCS, took more risks during travel needs to be studied further. Perhaps their higher degree of medical knowledge gave them a sense of false security instead of making them more conscious of risks encountered.

Pooled data from Paper II and III show that just over half (60%) of all travellers changed their behaviour to reduce the risk of accidents and/or crime. It is not known why all travellers did not do this. Some travellers may have made behavioural changes unknowingly. Research-based advice regarding personal security is scarce [222] and is difficult to generate. Evidence-based advice does exist [30, 222-225] and needs to be utilised by travellers. Accidents, mainly traffic accident and drowning, are one of the leading causes of death in travellers, especially in younger travellers [29-33, 36, 223]. In Paper III, 59% of students stated that they had felt concerned for their personal security once or repeatedly during their trip, further stressing the importance of including this topic in the pre-travel information.

The number of students in Paper III who met a new sex partner during travel was higher than what was found in Paper II (20% compared to 9%), but when comparing with travellers <30 years of age in Paper II, the proportions were more similar (20% compared to 15%). Our results correspond to levels found in other studies [37, 60]. The level of condom use in the two studies (53% and 65%) could possibly be increased if more travellers brought condoms with them during travel; only 25% of students in Paper III did that. However, a 2011 Swiss study found that supplying condoms to travellers did not reduce the level of unprotected sex [226].

Results from the AUDIT-C questions revealed that one-third of travelling students were in the category of risky drinking while abroad (36%), similar to the level in the Swedish population of corresponding age [227]. Risky drinking was significantly associated with being the victim of theft and meeting a new sex partner. A study on travellers to Peru showed that high alcohol consumption was linked to higher likelihood of using illicit drugs as well as meeting a new sex partner [39]. Being in an unfamiliar environment increases the risks associated with high alcohol consumption, another fact that travellers need to know. Questions on alcohol consumption were not included in study II, so a comparison between the study groups could not be made. The level of risky drinking in a group of Swiss travellers under 35
years was 15% during travel [40], although this number was derived using different methodology than what was used in study III.

**Paper IV – ESBL-PE colonisation in healthcare students**

In total, 35% of study subjects became colonised with ESBL-PE while travelling. All ESBL-PE isolates detected were ESBLA-positive *Escherichia coli*. No CPE were found in any sample despite extensive screening. It seems that CPE colonisation in travellers is still very rare [22, 228]. The ESBL-PE isolates also showed resistance to other antibiotics; for example, they were commonly found to be resistant to trimethoprim/sulfamethoxazole and ciprofloxacin (72% and 56% respectively).

Travel destination was the strongest risk factor for colonisation with ESBL-PE. Two-thirds of travellers to the South-East Asia region became colonised (67%) compared to 10% of travellers to the African region and 0% for the remaining destinations. This finding is consistent with regional differences found in other studies on travellers [20-25]. The other significant risk factor for colonisation found in Paper IV was antibiotic treatment during travel, a finding also in recent Finnish and French studies [24, 26]. In Paper IV, one in five study subjects were treated with antibiotics while travelling. Previous survey studies have shown levels of antibiotic treatment during travel between 1.5% and 9% [42, 48, 51]. The higher level found in our study was probably linked to the high incidence of travellers’ diarrhoea.

Unlike other studies [18, 20, 24, 25], our study found that travellers’ diarrhoea was not a risk factor for ESBL-PE colonisation. This difference may be explained by the fact that our study was not powered enough to detect this effect since so many study subjects suffered from travellers’ diarrhoea. Avoiding travellers’ diarrhoea is difficult as discussed previously [48, 52, 71, 72, 74-77] and it has also been difficult to find protective measures that reduce the risk of ESBL-PE colonisation during travel [22, 24, 25]. The best advice to travellers and travel medicine practitioners alike for reducing the risk for ESBL-PE colonisation is to avoid unnecessary antibiotic treatment while travelling [229].

Colonisation is not the same as infection. The consequences for a healthy individual of being colonised with ESBL-PE are probably small. Six months after travel, the colonisation level drops drastically (to 5%-9%) [21, 25, 26]. The individual risk for a symptomatic infection in someone colonised with ESBL-PE in the gut is difficult to assess. No ESBL-PE infection was recorded during a one-year follow-up of 90 Finnish travellers colonised after travel [24]. However, recent international travel has repeatedly been shown to be a
major risk factor for infections caused by ESBL-PE [134, 152-156]. Prolonged duration of colonisation with ESBL-PE has been seen in hospitalised patients [230], and faecal colonisation with ESBL-PE as well as KPC in hospitalised patients was a risk factor for subsequent bloodstream infections [231-233]. Quantifying the proportion of ESBL-PE in the gut microbiome might improve the evaluation of the risks with colonisation.

Ruppé et al. has quantified the abundance of ESBL-producing Enterobacteriaceae compared to all Enterobacteriaceae in the gut microbiome (Relative Abundance, RA) by using serial dilutions and counting colony-forming units (CFUs) on agar plates with or without cefotaxime [234]. This method found a correlation between level of RA and the likelihood of contracting a urinary tract infection with the same ESBL clone. The correlation was strongest between low RA and no infection with a 100% negative predictive value. A 57% positive predictive value for RA >10% was also found. A 13-fold increase in RA was seen following antibiotic treatment.

Using the same method, a prospective study of travellers [26] found a significant difference between mean RA and travel destination; travellers to Asia had higher mean RA than travellers to Sub-Saharan Africa and Latin America. The same study found that a higher mean RA was related to longer duration of colonisation by ESBL-PE after travel. These results show that quantifying the proportion of ESBL-PE indeed helps assess the risk with colonisation. Because the method used is time consuming, it is currently not suitable for routine clinical use. A higher positive predictive value would also increase the benefits of the method in the clinical setting.

Although the consequences of colonisation with ESBL-PE for the individual are difficult to evaluate, the consequences on the societal level are easier to quantify. The pre-travel colonisation rate found in study IV was 7%. Community carriage rates of ESBL-PE in Swedes in studies performed in 2007 and 2009 were 1% and 2.4%, respectively. This indicates a clear increase in carriage rates in less than a decade. In Sweden, all ESBL-PE diagnosed in laboratories are notifiable to the Public Health Agency. In 2007, 2098 cases of ESBL-PE were reported to the Public Health Agency and in 2014 the number increased to 8901 cases [151]. Although difficult to prove a causative relationship, with nearly 13 million international departures from Swedish airports in 2014 [235], the importation of ESBL-PE in the gut of travellers is likely a contributing factor to the increase in ESBL-PE infections in Sweden. Although an ESBL-PE colonised healthy individual traveller has a low probability of becoming sick, the collective impact of travellers may contribute significantly to an overall increase in symptomatic infections. At the society level, there will be events when ESBL-PE are shared between
individuals after return from travel, e.g. in families, and family members with underlying disease may be the ones experiencing ESBL-PE infections.

We did not find that professional healthcare exposure abroad was related to an increased risk of ESBL-PE colonisation. This has not been studied previously. Professional healthcare exposition abroad does therefore not in itself warrant screening of healthcare students and healthcare workers. However, based on the increasing community carriage level and the fact that up to 70% of travellers to certain geographical areas are colonised following travel, screening of healthcare personnel for ESBL-PE in a more general fashion can be discussed. Having a family member with an infection caused by ESBL-PE has been shown to be a risk factor for ESBL-PE colonisation [168, 169], indicating transmission between household contacts. Transmission between ICU patients of ESBL-producing *Klebsiella pneumoniae* have been reported [236], but no studies describing transmission of ESBL-PE from colonised staff to patients could be found. None of the staff members were found to be carrying the outbreak strain in an extensive investigation into a three-year outbreak of ESBL-producing *Klebsiella pneumonia* in a neonatal ICU in Germany [237]. Since ESBL-PE is mostly carried in the gut, proper hand washing procedures together with the practice of standard hygiene precautions makes the risk for transmission of ESBL-PE from colonised healthcare personnel very low. Therefore, the screening of hospital staff for carriage of ESBL-PE is currently not recommended.

**Paper V – Travel-related changes in the gut microbiome**

The metagenomic sequencing revealed that the majority of resistance genes and the overall bacterial composition of the gut microbiome remained unchanged following travel to the Indian peninsula and Central Africa. Significant increases were found in less abundant but clinically important resistance gene categories, namely genes encoding resistance to tetracyclines (1.04-fold increase), aminoglycosides (1.5-fold increase), beta-lactams (2.6-fold increase), sulphonamides (2.6-fold increase), and trimethoprim (7.7-fold increase). Significant increases in the mobile elements integrases (intI10) and ISCR2 (Insertion Sequence Common Region) were also seen. When investigating individual resistance genes, 178 resistance genes were found in total among all 35 samples and nine genes showed a significant increase after travel. Although rare in absolute counts these genes included beta-lactamases (DHA and TEM) as well as genes encoding resistance to trimethoprim (*dfrA* variants) and tetracyclines (*tet(B)*).
The increase in resistance genes was most prominent in samples from travellers to Central Africa, but there were no significant differences in resistance gene abundance between travellers to Central Africa and travellers to the Indian peninsula. The increase in resistance genes was not related to a number of risk factors tested, including travellers’ diarrhoea, professional hospital exposure, and the use of malaria chemoprophylaxis during travel. Since none of the study subjects had taken antibiotics, all recorded changes occurred without antibiotics affecting the gut microbiome.

The ESBL-PE screening showed that 12 of 18 travellers returning from the Indian peninsula were positive, but none returning from Central Africa were positive. Sequencing of the ESBL-PE positive isolates showed that all isolates carried the CTX-M-15 gene, a quite expected result since it is the dominating genotype in India [238]. This gene however, was not detected in the majority of corresponding sequenced metagenomes. So despite the sequencing depth used (detecting a resistance gene present in one out of approximately 100,000 bacterial cells), all clinically important genes were not detected. However, several ESBL-genes were detected by the metagenomic sequencing in study subjects with a negative ESBL screening test (Paper V, Figure 4). This finding might be due to sensitivity shortcomings in the culture screening procedure on chromogenic media, or because these genes existed in non-cultivable species or in non-Enterobacteriaceae species (only ESBL-PE isolates were sequenced).

The reason for the increase in ESBLs and other resistance genes following travel is unknown. Potential explanations are the enrichment of resistant bacteria already present in the gut, the replacement of susceptible bacteria with resistant bacteria, and the acquisition of resistance genes from a transient gut microbiome. Several mechanisms are most likely involved at the same time. Antibiotics exert a selection pressure favouring bacterial strains harbouring resistance genes already present in the gut microbiome, but they also disrupt the colonisation barrier in the gut allowing for a replacement with more resistant bacteria [239, 240]. Acquisition of resistance genes may occur through ingestion of resistant bacteria in food [241] or water [242-244].

The stability of gut microbiomes over time has been established [147, 148], so it was not surprising that we did not find major changes to the taxonomic composition following a period of travel. The phylum Proteobacteria (constituting on average 4% of the human microbiota), however, showed a significant increase following travel. Proteobacteria contains several clinically important bacterial families such as Enterobacteriaceae. No significant changes were seen on this taxonomic level although a non-
significant increase of the *Escherichia* genus in travellers to Central Africa was seen. The increase in Proteobacteria was not significantly correlated to the increase in resistance genes, but a larger sample set is needed to determine whether there truly is no connection between the increase in Proteobacteria and the increase in resistance genes. A connection between the two theoretically makes sense in light of the increase of ESBL-PE following travel and would represent an uptake of resistance genes in pathogenic bacteria during travel.

Travellers’ diarrhoea might be a driver behind the increase in Proteobacteria during travel, although we did not find such an association. Proteobacteria has been associated with *Clostridium difficile* enteritis [245] and *Clostridium difficile* is, like TD, a bacterial infection of the large intestine. Several previous studies have shown a relationship between TD and ESBL-PE colonisation during travel. We did not find this relationship in Paper IV, perhaps because of the high incidence of TD in relation to the number of study subjects. This too would preferably be studied in a larger cohort of study subjects.

Co-localisation network analysis showed that many of the significantly increased genes were localised together on the same assembled DNA fragment (Paper V, Figure 5); this included genes encoding resistance to aminoglycosides and macrolides. The resistance genes were in some cases found together with ISCRs and integrases, indicating mobility potential. The assembled DNA fragments revealed that it was not possible to see in which bacterial species the resistance genes were located. This would be very interesting to study but seems difficult with the shotgun sequencing used in this study.

In the future, linking resistance genes with bacterial species in microbiomes could be achieved by analysing Hi-C data. With the Hi-C method, cells are cross-linked with formaldehyde. A restriction enzyme digests the DNA and the fragments are then ligated and marked with biotin at the junctions. The DNA sample thus contains fragments that originally were in close proximity. After shearing, the biotin-containing fragments are selected with the help of streptavidin beads and sequenced by massive parallel sequencing. Preliminary results using the Hi-C method on a simple synthetic metagenome showed that it was possible to assemble almost complete bacterial genomes. This enables associating resistance genes with bacterial species. The preliminary results also showed that plasmids could be linked to the chromosomes of their bacterial host and with each other thereby allowing detailed study of mobile genetic elements within bacteria [246-248].
Conclusions

There are sex-related differences in acceptance of travel vaccinations, but the reasons for these differences are unknown. Clearly, more research is needed to find the reasons for and consequences of this difference. Travel medicine practitioners need to be aware of this phenomenon when advising travellers.

Compliance with travel advice had no effect on the incidence of health problems during travel and younger travellers especially need travel advice that is better matched to the risks they face when abroad. A comprehensive review of available travel advice is needed. Not giving any advice is not recommenced since most risks during travel cannot be prevented by vaccination and prophylactic medication. Given our results, the pre-travel advice should focus on malaria and compliance with chemoprophylaxis, accidents, personal security issues, dangers with excessive alcohol use, unprotected casual sex, and the consequences of unnecessary use of antibiotics during travel.

Study abroad students had higher incidence of health problems than tourists. Healthcare students are exposed to more travel-related risks than non-healthcare students and also expose themselves to greater risks through risky behaviour despite receiving more travel advice than non-healthcare students. These risks need to be targeted in the pre-travel preparation of healthcare students studying abroad.

ESBL-PE colonisation was found to be very common in travellers to certain geographical areas, especially the South-East Asia region. Antibiotic treatment was an independent risk factor for colonisation. Professional healthcare exposure abroad was not related to an increased risk for ESBL-PE colonisation. General post-travel screening for ESBL-PE of healthcare students and healthcare workers cannot currently be motivated.

Metagenomic sequencing provides a novel method for studying the geographic spread of antibiotic resistance and revealed a high diversity of resistance genes being enriched in the gut microbiome during travel. A drawback as compared with standard culture screening for ESBL-PE was a lower sensitivity for certain ESBL genes. A larger cohort of study subjects and new methods to better characterise resistance gene distribution in the microbiome are needed to further investigate the effects of travel on resistance gene abundance.
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