Unwanted souvenirs

*Travel-related acquisition of antibiotic-resistant Enterobacteriaceae and enteric pathogens*

van Hattem, J.M.

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General discussion and future directions
Antibiotic resistance and travel

The results of the studies presented in the first part of this thesis provide evidence that extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) are frequently being acquired during travel and imported into the Netherlands by travellers. Travel destination, especially travel to countries in Asia and Northern Africa, is the most important predictor. Other important predictors for ESBL-E acquisition during travel are antibiotic use during travel, traveller’s diarrhoea (TD), particularly if persisted on return, and pre-existing chronic bowel disease. A substantial proportion of travellers carry ESBL-E for prolonged durations after acquisition and household transmission does occur. The data upon which these conclusions are based were largely drawn from studies presented in Chapter 3 of this thesis. Additionally, we have shown acquisition of plasmid-mediated carbapenemase and colistin resistance encoding genes in the same study cohort (Chapters 4-6).

Travel-related acquisition of ESBL-E

The importance of travel destination

In concordance with previous studies [1], more than one third of all travellers in our cohort of travellers acquired ESBL-E. The risk of ESBL-E acquisition varied widely between subregions and countries. ESBL-E were most frequently acquired in Southern Asia (75.1%, 95% CI 68.4–80.9) with acquisition being highest among those who had travelled to India (88.6%, 95% CI 79.8–93.9). These numbers are in sharp contrast to acquisition in travellers to Southern Africa, where only 7 of 116 travellers (6.0%) acquired ESBL-E. The reason for the large differences in acquisition rates between regions is most likely multifactorial. The factors that are potentially involved are discussed below.

First, the local prevalence of antibiotic resistance in the community is a major and biologically plausible factor that increases the risk of travellers coming into contact and acquiring resistant bacteria.

Asia is well known, if not notoriously so, as one of the epicentres of antimicrobial drug resistance [2]. A review on the epidemiology of multidrug-resistant (MDR) Escherichia coli in Asia has shown the prevalence of CTX-M–producing E. coli to be very high [3]. Resistance in most parts of Asia is no longer limited to patients that have had contact with health care but has likely spread through the community including rural areas [3, 4]. Although possibly biased towards a population that is more prone to acquisition and...
carriage of ESBL-E, a study demonstrated that >70% of clinical E. coli isolates collected from community-acquired intra-abdominal infections in India were ESBL producers [5]. A population based study to assess the prevalence of antibiotic resistance in E. coli and Klebsiella pneumoniae isolated from patients with urinary tract infections (UTIs) on two islands of Indonesia showed that >50% of community based isolates were resistant to 3rd-generation cephalosporins [6]. Of note, Indonesia was the most popular travel destination in our study and was visited by 211 travellers of whom 19.0% acquired ESBL-E.

A systematic review on antibiotic resistance among isolates in sub-Saharan Africa that included studies published between 1996 and 2012, showed a median prevalence of resistance to 3rd-generation cephalosporins, presumably largely due to the production of ESBLs, of 0.0-46.5% in Enterobacteriaceae [7]. Another systematic review that included studies published from 2005 onwards found that the proportion of ESBL-E from infected or colonized patients is higher in Northern than in Southern Africa (12.5% VS 8.3%) [8]. In a study from Egypt, which included only blood stream infections in patients in intensive care units (ICUs) that were admitted in 2006 and 2007, the proportion of ESBL-E exceeded 75% [9]. Also, relatively high proportions of ESBL-producers of up to 31.4% were found in clinical isolates in studies in Algeria and up to 20.2% and 7.5% in neighbouring countries Tunisia and Morocco [8]. On the other hand, the proportion of ESBLs was extremely low (0.7%) in Enterobacteriaceae that were isolated from blood cultures taken in 2004 and 2005 in a study from Malawi [10]. Since most of this data comes from clinical studies, carriage rates in the community, where travellers contract ESBL-E, may be lower. A surveillance study on susceptibility patterns of 358,843 E. coli strains isolated from urine samples in South Africa from 2007-2011 found 8% of the isolates to be ESBL-positive, a prevalence that is comparable to northern European countries [11]. This low prevalence may explain the low acquisition rates of 4.6% and 6.0% in travellers to South Africa and the subregion Southern Africa respectively in our travellers.

Multidrug-resistant Enterobacteriaceae are also a concern in South America where they are found in both nosocomial and community-acquired infections, even in remote communities [12, 13]. In the SENTRY Antimicrobial Surveillance Program in Argentina, Brazil, Chile and Mexico, ESBL rates of gram-negative bacilli, that were recovered from hospitalized patients with serious community- or hospital-acquired infections between January 2008 and December 2010, were 18%, 13%, 24%, and 48% among E. coli and 60%, 50%, 59%, and 33% among Klebsiella spp. respectively (overall 25% for E. coli and 53% for Klebsiella spp.) [14]. Another study in 10 Latin American countries found 27% of E. coli and 38% of K. pneumoniae collected from intra-abdominal infections to be carriage of ESBL-E, a study demonstrated that >70% of clinical E. coli isolates collected from community-acquired intra-abdominal infections in India were ESBL producers [5]. A population based study to assess the prevalence of antibiotic resistance in E. coli and Klebsiella pneumoniae isolated from patients with urinary tract infections (UTIs) on two islands of Indonesia showed that >50% of community based isolates were resistant to 3rd-generation cephalosporins [6]. Of note, Indonesia was the most popular travel destination in our study and was visited by 211 travellers of whom 19.0% acquired ESBL-E.

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ESBL positive [15]. However, a large surveillance study in community-acquired UTI's in Curitiba, a large southern Brazilian city, in 2009 found only 3% of E. coli to be resistant to 3rd-generation cephalosporins [16]. These data could reflect a high prevalence in health care and a relatively low prevalence in the community, which could account for the relatively low acquisition rate of 8.0% in travellers to Brazil in our study.

Second, low hygiene standards at the travel destinations contribute to local dissemination of resistant bacteria and increase the chance of acquisition by travellers. In the absence of proper sanitation, e.g. if good sewage systems are lacking, resistance genes can become widely disseminated in the environment, potentially contaminating surface and drinking water. Subsequently, crops and food products may become contaminated by irrigation or washing with contaminated water. As a consequence, the local population and visiting travellers are at risk of contracting ESBL-E through contaminated water or food. Since Enterobacteriaceae are gut colonizers, they are directly or indirectly transmitted through the faecal–oral route [17, 18]. In low- and middle-income countries, proper sanitation that can act as an effective barrier to stop transmission of bacteria via faecal–oral pathways [Figure 1] is often limited, allowing bacteria to easily spread between people, including direct human-to-human transmission to travellers.

In conclusion, the prevalence of EBSL-E differs significantly between subregions and countries within subregions. Indeed, in Asia, Northern Africa and Southern Africa ESBL-E prevalence seems to be correlated with likelihood of acquiring ESBL-E by Dutch travellers. In addition to local prevalence, a difference in hygiene standards could account for the difference in acquisition rates between travel destinations.

Other risk factors for acquisition of ESBL-E and possible interventions

International tourist arrivals have increased from 25 million globally in 1950 to 278 million in 1980, 674 million in 2000, and 1.2 billion in 2016. This number is estimated to reach 1.8 billion by 2030 according to the World Tourism Organization's (UNWTO) long-term forecast report ‘Tourism Towards 2030’, many of whom arriving from emerging economies [Figure 2] [20]. Discouraging people to travel or to limit travel to areas at high risk for the acquisition of resistant bacteria obviously is unrealistic.

Although there are several initiatives to combat resistance such as the ‘Global action plan on antimicrobial resistance’ as released by the WHO in 2015, it is unlikely that they will have impact in the short term. Therefore, one of the objectives of this thesis was to identify determinants of acquisition that are suitable for an intervention in order to prevent acquisition of resistant bacteria during foreign travel.
Figure 1. Diagram showing pathways of faecal-oral disease transmission. [19]
The vertical blue lines show barriers to disease transmission: toilets, safe water, hygiene & handwashing. This scheme comes from a UNICEF commissioned children’s storybook that has still not been printed and published. The language is in English and Filipino.
Important predictors for acquisition identified in our studies were antibiotic use during travel, bowel disease and the occurrence of TD, particularly TD that persists upon return. These risk factors do not directly lead to an increased exposure, but may make the host more susceptible to acquiring resistant bacteria by decreasing colonization resistance of the gut [21]. In addition, antibiotic use could lead to selective growth of resistant bacteria after acquisition or improve survival of bacteria that acquired resistance genes by horizontal gene transfer. Future research is needed to study changes in microbiota caused by travel in general and by these risk factors specifically and how these changes predispose for acquisition. Another predictor was the consumption of food from street vendors in the general population. In the separate analyses of three of the visited subregions (Southeastern Asia, Southern Asia and Eastern Africa) risk factors that were specific for each subregion were found. Interestingly, most of them concerned behaviour during travel that could have led to an increased exposure to ESBL-E, such as food consumption, contact with orphan children and staying in rural areas (Chapter 3). Avoiding these risk factors by travellers would, in theory, help to minimize influx of resistance into the Netherlands.

In our study, 132 travellers (7.2%) used antibiotics during travel of whom 73 (55.3%) acquired ESBL-E, compared to 32.6% of travellers who had not used antibiotics. However, since only a small proportion of travellers used antibiotics during travel, the attributable proportion of antibiotic use to total acquisition is calculated to be 0.05, i.e. about 5.0% of the total ESBL-E acquisition in our cohort was attributable to antibiotic use during travel. Pre-travel advice on limiting the use of antibiotics to very specific clinical situations during the pre-travel consultation, as suggested by some [22-24], would thus only have a limited effect on reducing the overall import of ESBL-E by travellers, although this effect might be higher in countries where antibiotic usage during travel is more liberally advocated. Certainly, this limited overall effect should not be a reason for advising against a restricted usage of antibiotics to travellers for self-limiting diseases such as TD since it will decrease the chance of acquiring resistant bacteria by individual travellers.

Since much more travellers are reporting TD than AB use during travel (7.2% VS 39.9%), prevention of TD would have a larger effect (attributable proportion 0.12), although prevention of TD e.g. by dietary precautions is notoriously difficult [25]. Even when TD would somehow have been prevented in every traveller within our cohort, still 30.3% of travellers would have acquired ESBL-E. Avoiding consumption of meals at street food stalls during travel (attributable proportion 0.096) by all travellers would reduce acquisition from 34.2% to 30.9%.

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The only significant protective factors against acquisition of ESBL-E that were found in our study were beach holidays and the attendance of large (religious) gatherings, possibly related to decreased exposure, but both not very useful in preventing travellers from acquiring ESBL-E. Interestingly, measures generally thought to be protective such as hand hygiene and following certain diets did not protect travellers from acquisition.

Minimizing risks will thus only have little effect on the import of ESBL-E by travellers overall and regardless of these factors, the import will remain substantial. Apparently, travellers contract ESBL-E and other multidrug-resistant bacteria during routine holiday activities. Possibly, determinants that have not been studied could account for the acquisition. One can only speculate on the as yet hidden sources and transmission routes of such strains, but acquisition by assumed ‘clean’ drinking water, showers, and toiletries, contaminated surfaces such as door handles, hand shaking, food and cutlery and even vector borne transmission are theoretically all possible [26]. More in depth studies into potential sources of multidrug resistant bacteria and their transmission routes to travellers in are needed.

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Transmission of acquired ESBL-E and spread into the community

Once imported by travellers into the Netherlands, the question is whether acquired resistant bacteria or resistance genes can spread in the community. In our study, sustained carriage after return from travel was observed in 11.3% of our travellers up to at least 12 months after return. Prolonged carriage increases the likelihood of transmission to others. In the mathematical model of onward transmission of ESBL-E in households of travellers, person-to-person transmission occurred at a rate of 0.0013 per colonized person per day and decolonization at 0.010/day (duration of colonization 100 days). The estimated probability of transmission from a traveller to a household contact was estimated to be 12%. If the transmission rate (0.0013/day) is multiplied with the average duration of carriage (100 days) and the average number of household members (1.54) the basic reproduction number $R_0$ - the number of secondary colonisations followed by one acquisition – is calculated to be 0.2. Although this has not been studied in our cohort, it is likely that transmission rates between households (i.e. from one household to another) are lower than within households.

As sustained spread in a population is reached only if the $R_0$ is above 1, this would imply that person-to-person transmission after travel-related acquisition is unlikely to substantially contribute to further spread of ESBL-E in the community. However, considering the very high and increasing number of international travels, the import of ESBL-E by travellers is and will continue to be substantial. Taking into account the total number of Dutch travellers visiting these regions annually, we have estimated that each year between 3.0% and 7.1% of the Dutch population acquires an ESBL-E during travel to destinations outside Europe, northern America, and Oceania. As part of these imported ESBL-E are sustainably carried and part will transmitted to others, travel-related acquisition of ESBL-E still contributes to at least a part of the background prevalence of ESBL-E in the Netherlands. Further community-based studies are needed to investigate to what extent this is the case and what the other contributors are.

A Dutch study that used the same model to quantify within-household transmission of ESBL-E acquired during hospitalization found a comparable duration of colonization of the healthy household members of 111 days, but the direct transmission rate was approximately 4 times higher (0.0053/colonized person/day) [27]. This difference might be due to an increased exposure to ESBL-E by caregiving household members of recently discharged patients compared to household members of returning travellers.

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Travel-related acquisition of carbapenemase-producing Enterobacteriaceae

In Chapter 4 we describe five travellers, all visiting Asia outside the Indian subcontinent, who acquired carbapenemase-producing Enterobacteriaceae (CPE). One traveller persistently carried the same OXA-244 CPE up to 6 months post-travel. Three months after travel, her co-traveling spouse also became positive for this OXA-244 CPE strain, suggesting clonal transmission within this household.

Even though CPE and their genes have disseminated in several parts of the world, and several prospective studies have studied acquisition of CPE in travellers, so far, only two other prospective studies have found acquisition of CPE in travellers. Two French travellers were colonized with OXA-181 and one with NDM-1 in after returning from India [28] and an OXA-48-producing *E. coli* was acquired by a Dutch traveller while visiting Egypt [29].

Large community reservoirs of carbapenemase genes exist, including NDM in Myanmar [30], India [31, 32] and Pakistan [33], OXA-48-like carbapenemases in Northern Africa [34], Turkey and Spain [35] and closer to home, KPC in Italy, Greece and Israel [35]. Large numbers of Dutch travellers visit friends and relatives in Morocco and Turkey each year and Italy, Greece, Turkey and Egypt are among the most popular holiday destinations [36]. Given the endemicity of carbapenemase genes in these countries and the large numbers of yearly visits, travellers to these countries might contribute even more to the import of CPE into the Netherlands than the relatively low number of travellers to Asia. Unfortunately, we were not able to include enough travellers to sufficiently study acquisition in travellers to these countries, since many of them do not visit travel clinics for pre travel advice and vaccinations. Prospective studies focussing specifically on travel-related acquisition of multiresistant bacteria in these countries are needed. Recruitment and inclusion of travellers for these studies could take place in travel agencies, mosques and community centres.

Although travel-related-acquisition of CPE by (Western European) travellers is limited, further spread into low-endemic countries such as the Netherlands is undesirable, hence screening for CPE in patients who are admitted to healthcare facilities after returning from high-risk countries should be considered. Based on our studies, such screening should at least include travellers who have travelled to Asia including the Indian subcontinent within the last month, but possibly should also include those who have travelled to Morocco, Turkey, Greece, and Egypt based on high local prevalence in these countries.
Global spread of the mcr-1 gene

Travel-related acquisition of mcr-1

In Chapter 5, we report the acquisition of the colistin resistance-conferring mcr-1 gene in six participants of our cohort who had travelled to 3 different continents. In another study, the mcr-1 gene was detected in faecal metagenomic DNA from 6 of 122 healthy Dutch long-distance travellers after they had visited destinations in South (east) Asia and Southern Africa between 2011 and 2012 [37]. In addition, acquisition of the mcr-1 gene during the Hajj (the Muslim pilgrimage to Mecca) in 2013 and 2014 has been reported [38]. Travel-related acquisition of mcr-1-carrying isolates suggests a global spread of the mcr-1 gene in the community.

Mechanisms of global spread of mcr-1

As shown in Chapter 6, such global spread of the mcr-1 gene is facilitated by efficient horizontal gene transfer dominated by a limited number of plasmid incompatibility types rather than the dissemination of one or more successful clones. The insertion sequence (IS) ISApl1, as described in the first publication on mcr-1 [39], is thought to be a key component in the mobilization of the mcr-1 gene. This IS is described as highly active and being able to transpose at a very high frequency in different nonspecific insertion sites [40] making it able to integrated into plasmids. Specifically, three mcr-1-carrying plasmids are successful, as more than 90% of all identified plasmids belonged to incompatibility groups IncX4, IncI2 or IncHI2.

In our study, the ISApl1 was only found in a minority of studied plasmids and completely missing in all of the 24 reported IncX4 plasmids. This could mean that the mcr-1 gene lost ISApl1 and stabilized it into the plasmid or the mcr-1 gene was transferred by other insertion sequences such as IS26/26-like [41, 42]. Two populations of mcr-1-carrying E. coli that could have contributed to the spread of mcr-1 were identified. Isolates within those lineages were recovered from different continents and carried a diversity of mcr-1-carrying plasmids, supporting the hypothesis of spread of MGEs rather than clones. Nevertheless, these E. coli populations could play an import role as a reservoir and vector for this gene due to their capability to colonize humans and food animals and possibly their ability of acquiring resistance genes or MGEs. Both lineages have been described as vectors of spread of resistance genes from animals to humans. In addition, a high proportion of isolates from both humans and animals was positive for the florfenicol floR (almost 50%) and novobiocin baeR and baeS (almost 100%) resistance.
genes. Florfenicol and novobiocin are used almost exclusively in veterinary medicine. Altogether, these findings support the hypothesis of an animal reservoir, that that is driven by the use of colistin in livestock, as a source of \textit{mcr-1} in humans.

**mcr-1 in the Netherlands**

In the Netherlands, \textit{mcr-1} was detected at low prevalence in \textit{E. coli} isolates from livestock (<1%)\cite{43}. However, the prevalence of \textit{mcr-1} as detected by PCR after overnight culture of retail chicken meat bought from supermarket chains throughout the Netherlands in 2015 was 24.8% (53/214 samples positive) of which 64.2% (34 of 53 PCR positive samples) were confirmed by selective culture \cite{44}. So far, carriage of \textit{mcr-1} positive bacteria in humans in the Netherlands has remained low. No \textit{mcr-1}-positive isolates were detected in a large collection of Enterobacteriaceae isolates of human origin from the Netherlands between 2009 and 2015 \cite{45}. A study that screened faecal samples of patients from a Dutch University Medical Center for the presence of \textit{mcr-1} using real-time PCR found 2 of 576 patients, without history of recent travelling or colistin use, positive for \textit{mcr-1}, resulting in a prevalence of 0.35% \cite{46}. Additionally, the \textit{mcr-1} gene was detected in 3 of 45 colistin-resistant \textit{E. coli} isolates from two patients \cite{47}. Whether these isolates are acquired in the community or nosocomial stays unknown.

**Conclusions**

In conclusion, the \textit{mcr-1} gene has disseminated worldwide in multiple reservoirs mainly through transfer of MGEs. Potentially, human travel has contributed to the dissemination of the \textit{mcr-1} gene, but phylogenetic analysis of \textit{E. coli} and plasmids carrying the \textit{mcr-1} gene indicates that the animal reservoir, and possibly the trade of animals and animal-derived food products, plays a more important role. The prevalence in clinical cultures in the Netherlands to date remains low. However, an increase in the use of colistin for treating infections with multidrug-resistant Gram-negative bacteria or the use of colistin for selective decontamination of the digestive tract (SDD) could select for \textit{mcr-1} carrying isolates, leading to an increase of colistin resistant isolates. Strict surveillance of colistin resistance in Enterobacteriaceae should be applied to those receiving colistin therapy and SDD.

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Travel-related acquisition of diarrhoeagenic pathogens

In Chapter 7, we describe the results of a pilot study in 98 travellers to determine the frequency of travel associated acquisition of 19 (entero)pathogens, including hepatitis E virus. The low acquisition rates of enteric viruses could indicate that the role of viruses as the cause of persisting TD is limited or that viral RNA or DNA is rapidly cleared before returning. Since bacterial pathogens were acquired more often, they are likely to be more important as a cause of TD. We found a substantial proportion of travellers carrying *Blastocystis* spp. and *Dientamoeba fragilis* before and after travel. A follow-up study on carriage before and after travel and geographic distribution of acquisition of different subtypes of *Blastocystis* in a larger subset of travellers (Chapter 8), confirmed that many travellers were already carriers before travel. Additionally, limited acquisition was found and, interestingly, in a substantial proportion of travellers that were positive before travel, no *Blastocystis* was detected post travel, indicating a high rate of loss.

Implication of findings

Implications for public health

The acquisition of multidrug resistant strains by travellers may pose a risk for the health of individual travellers and be transmitted to their household members and, perhaps less likely, may contribute to spread in the community. In addition, transmission and spread within health care settings when travellers present themselves to health care in the months following their return could occur. Currently the expectation is that no simple measures can be taken to prevent the acquisition of antibiotic-resistant organisms during travel and, therefore, the influx will continue. Probably this travel-related import of antibiotic resistance has already been ongoing for years.

Although prevalence is slowly increasing, countries in the northwest of Europe, including the Netherlands, nevertheless have kept resistance among isolates of *Enterobacteriaceae* in the community to a more or less acceptable level. Indeed our data suggests that transmission rates after travel-related acquisition likely are not high enough to lead to endemicity in the Netherlands in the foreseeable future. However, travel-related acquisition inevitably contributes to the background prevalence. Therefore the increasing number of international travels and transmission from livestock, food products and the environment together could account for the increasing background prevalence.
Implications for clinical care

For the choice of empirical therapy in patients that are diagnosed with severe infections caused by Enterobacteriaceae, such as complicated urinary tract infections (UTIs), peritonitis, cholangitis and intra-abdominal infections in the hospital setting, it is common practice to take into account results of previous bacterial cultures if available. Patients that are known carriers of ESBL-E often will often be empirically treated with carbapenems.

We found that travel to certain countries is associated with high acquisition rates and subsequent carriage of ESBL-E, especially shortly after return. In cases where patients are admitted with severe infections shortly after travel to high-risk destinations, the chance of carriage of ESBL-E is so high that an adjustment of the empirical therapy is justified. Also, for infection prevention purposes in health care facilities, we should consider screening these patients for carriage of multidrug-resistant gram-negative bacteria and take appropriate isolation measures until screening results are available. At least, this should be the case in low prevalent countries such as the Netherlands. Patients with low risk of carriage and mild to moderately severe infections can perhaps be treated according to current guidelines, preserving last resort antibiotics for those who need it.

In the outpatient setting, e.g. in returning travellers presenting themselves to the general practitioner with uncomplicated or complicated UTIs, the risk of travel-related acquisition of resistant Enterobacteriaceae could likewise be relevant. If infections are caused by resistant micro-organisms, empirically prescribed oral therapies might fail. It is therefore advisable, to perform cultures, e.g. from urine, in case of infections after recent travel to Asia, Northern Africa and possibly the Mediterranean countries before treatment, but certainly in case of failure of first line therapies.

Above considerations in returning travellers should be taken into account in guidelines for antibacterial therapy (e.g. sepsis and complicated UTIs) and infection prevention.

Implications for individual travellers

In addition to optimizing empiric treatment of infections in travellers seeking medical help after travel, avoiding risk factors during travel could minimize the risk of acquiring resistant bacteria during travel. Pre-travel advice should focus on prevention of TD, e.g. by not eating raw vegetables or food from street vendors, and on limiting AB-use during travel. As mentioned before, these measures will have only a marginal impact on the overall acquisition of ESBL-E among travellers, but will decrease the risk of acquisition for the individual traveller.

We found that travel to certain countries is associated with high acquisition rates and subsequent carriage of ESBL-E, especially shortly after return. In cases where patients are admitted with severe infections shortly after travel to high-risk destinations, the chance of carriage of ESBL-E is so high that an adjustment of the empirical therapy is justified. Also, for infection prevention purposes in health care facilities, we should consider screening these patients for carriage of multidrug-resistant gram-negative bacteria and take appropriate isolation measures until screening results are available. At least, this should be the case in low prevalent countries such as the Netherlands. Patients with low risk of carriage and mild to moderately severe infections can perhaps be treated according to current guidelines, preserving last resort antibiotics for those who need it.

In the outpatient setting, e.g. in returning travellers presenting themselves to the general practitioner with uncomplicated or complicated UTIs, the risk of travel-related acquisition of resistant Enterobacteriaceae could likewise be relevant. If infections are caused by resistant micro-organisms, empirically prescribed oral therapies might fail. It is therefore advisable, to perform cultures, e.g. from urine, in case of infections after recent travel to Asia, Northern Africa and possibly the Mediterranean countries before treatment, but certainly in case of failure of first line therapies.

Above considerations in returning travellers should be taken into account in guidelines for antibacterial therapy (e.g. sepsis and complicated UTIs) and infection prevention.

Implications for individual travellers

In addition to optimizing empiric treatment of infections in travellers seeking medical help after travel, avoiding risk factors during travel could minimize the risk of acquiring resistant bacteria during travel. Pre-travel advice should focus on prevention of TD, e.g. by not eating raw vegetables or food from street vendors, and on limiting AB-use during travel. As mentioned before, these measures will have only a marginal impact on the overall acquisition of ESBL-E among travellers, but will decrease the risk of acquisition for the individual traveller.
**Future perspectives**

Roughly, two factors likely account for an increased risk of acquisition of ESBL-E in travellers: increased exposure to ESBL-E and a decrease in colonisation resistance of the gut due to dysbiosis caused by chronic bowel disease or travel-related factors, such as TD, antibiotic use or simply the alteration of gut microbiome due to the travel itself [21]. To determine exactly what sources are responsible for acquisition of resistant bacteria by travellers, local research at the travel destination is needed, e.g. by sampling food products and the environment. Future research is also needed to study changes in microbiota in relation to the risk factors that are found in this thesis and to travel in general and if and how these changes predispose for acquisition.

Risk factors for sustained carriage could be strain-, plasmid- and host-related. To determine whether travellers persistently carry the same strain or MGE only and to determine if strain factors are responsible for the sustained carriage, a follow up study comparing whole genome sequences (WGS) of shortly and persistently carried isolates is needed. Community-based studies could further quantify the role of travel-related acquisition of resistant bacteria to community carriage and other factors, such as the role of the human-animal interface and the environment.

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