Unwanted souvenirs

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

van Hattem, J.M.

---

**Citation for published version (APA):**

---

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

---

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
General introduction
Antimicrobial and antibiotic resistance

Antimicrobial resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases [1]. Antimicrobial resistance refers to resistance in microorganisms in general, including bacteria, viruses, fungi and parasites and therefore encompasses resistance to all antimicrobials, i.e. antibacterial, antiviral, antiparasitic and antifungal drugs. Since bacterial infections are treated with antibiotics, the term ‘antibiotic resistance’ is specifically used to indicate resistance in bacteria.

Infections with resistant bacteria can no longer be treated by first-line antibiotics, potentially leading to decrease in therapeutic outcomes, more side-effects and toxicity, and an increase in health care costs. Organ transplantations, chemotherapy and surgery will carry a much higher risk of infectious disease-related morbidity and mortality without effective antibiotics for the prevention and treatment of bacterial infections [3].

The increased and often inappropriate use of second- or third-line therapies accelerates the development of resistance against these agents. The vicious circle of using newer and broader spectrum antibiotics and the development of resistance against these has already led to pan-resistant microorganisms. Some say that because of resistance we are potentially returning to the pre-antibiotic era where people will die again from previously easy to treat infections because of resistance [4]. The lack of development of novel antibiotics necessitates the conservation of existing ones by implementing strict stewardship programs and controlling the spread of resistance [5, 6]. To be able to stop resistance from spreading, insight in the determinants of the emergence and spread of antibiotic resistance is needed.

The first part of this thesis focusses on resistance in Enterobacteriaceae, a family of gram-negative bacteria. First, emerging antibiotic resistance and its drivers, including import by travellers, are discussed. Next, some important resistance mechanisms in Enterobacteriaceae are highlighted followed by the possible implications for health care and the aims and outlines of the thesis. For the second part of this thesis, travel-related acquisition of viral, bacterial and parasitic diarrhoeagenic agents and hepatitis E virus is studied, with a focus on Blastocystis spp..
Antibiotic resistance and its drivers

Global and local emergence of antibiotic resistance

Over the last decades, the prevalence of bacteria that are resistant to antimicrobials has increased worldwide, including in the Netherlands [7] (Figure 1). Antibiotic resistant isolates are not only found in patients that have contact with health care but have also become increasingly prevalent in the community [8].

Since most resistance mechanisms are associated with fitness costs (i.e. reduced bacterial growth rate compared to wild type strains in the absence of antibiotics), most resistant bacteria will only persist in the presence of selective antibiotic pressure [9]. However, mutations that compensate for fitness losses without affecting resistance levels may occur, leading to clones that are as fit as susceptible ones and are able to survive in the absence of antibiotic pressure [9].

At any rate, antimicrobial use is one of the most important drivers of antibiotic resistance. The inappropriate use of antibiotics in human medicine has led to high levels of resistance in parts of Asia, Northern Africa and southeastern Europe [11-13]. Inappropriate antibiotic usage is also common in livestock and in agriculture and resistant micro-organisms are not exclusive to humans but also exist in animals, in foodstuffs and in the environment.

The global emergence of resistance is further aggravated by the national and international spread of resistance clones that are locally selected for, as shown by the global spread of the extra-intestinal pathogenic Escherichia coli (ExPEC) clone, ST131 [14]. Through human travel, food and animal trade, and through the consumption of meat, vegetables and fruit, all these reservoirs interact on a worldwide scale (Figure 2). We therefore have to address the emergence of antibiotic resistance from a One Health perspective [15].

To determine which intervention strategies are most effective in combating antibiotic resistance, it is important to distinguish which fraction of the total burden is attributable to different sources, including animal reservoirs and vehicles such as foods or the environment [16, 17].
Figure 1. Percentage of isolates with resistance to third-generation cephalosporins, by country, EU/EEA countries [10]
Large volumes of antibiotics are used in food-producing animals, not only for prevention and treatment of infections, but also as growth promoters [19]. In fact, most antibiotics are used in animal husbandry [Figure 3] [2]. We know that the livestock industry holds a significant reservoir of antimicrobial-resistant Enterobacteriaceae with potential public health implications [20].

Since long, it has been hypothesized that resistance is transmitted from animals to humans, either through direct, whole-bacterium transmission or via the transmission of mobile genetic elements (MGEs). Based on the similarity of resistance genes, plasmids and bacterial multilocus sequence typing (MLST) of *E. coli* isolates derived from poultry, poultry meat, and human infections, there is evidence for a clonal transmission and dissemination route [21-23]. However, a study performing whole-genome sequencing on a selection of presumably related strains from one of the former studies, demonstrated that these isolates were not phylogenetically related [24, 25]. Therefore, the question whether resistant Enterobacteriaceae that cause human
infection originate from food-producing animals is still under debate and additional research is needed to determine the role of MGEs in transmission of antibiotic resistance determinants at the human-animal interface [26].

Resistance and the environment

The importance of the environment in the spread of antibiotic resistance is increasingly recognized [17]. Antibiotic use in humans, animals and agriculture selects for resistant microorganisms and resistance genes that can subsequently spread to the environment. Human and animal effluents continuously contaminate surface water and soil either direct or through wastewater. In addition, water and organic fertilisers used on food crops may disseminate drug-resistant bacteria in the food chain [27]. The environment, therefore, contains a huge pool of resistance genes that can potentially spread to humans [28]. To what extent the environment contributes to human exposure, compared to the clinical and veterinary or agricultural domains, needs to be further quantified [17].

Import and spread of resistance by travel

In addition to animals and the environment, the human microbiome, especially the microbiome present in the human gut, also contains resistance genes, albeit at relatively low densities; the fraction of resistance genes among all genes in the gut is estimated to be approximately 0.002 [29]. Thus, by human travel, pools of antibiotic resistance genes are also traveling. According to the World Tourism Organization (UNWTO), international tourist arrivals have increased from 25 million globally in 1950 to 278 million in 1980, 674 million in 2000, and 1,235 million in 2016 [30]. These numbers do not include business and other travel. Even at the estimated low densities of resistance genes in the indigenous human microbiome, international travel at such scale entails the movement and shedding (primarily by defaecation) of huge numbers of resistance genes from one country to another. In addition to the role of (inappropriate) antimicrobial use in humans and animals, the increase of international travel may, thus, also contribute to the worldwide emergence and dissemination of antibiotic resistance since it allows resistant clones or MGEs to be rapidly transported between regions [31]. With multidrug-resistant Enterobacteriaceae being highly prevalent in the community in many parts of the world, especially in Asia [11], Africa [32] and South America [33], there is a huge potential of acquiring and transporting bacteria and/or MGEs, for example from high to low endemic countries, such as the Netherlands, by international or intercontinental travel.

Resistance and the environment

The importance of the environment in the spread of antibiotic resistance is increasingly recognized [17]. Antibiotic use in humans, animals and agriculture selects for resistant microorganisms and resistance genes that can subsequently spread to the environment. Human and animal effluents continuously contaminate surface water and soil either direct or through wastewater. In addition, water and organic fertilisers used on food crops may disseminate drug-resistant bacteria in the food chain [27]. The environment, therefore, contains a huge pool of resistance genes that can potentially spread to humans [28]. To what extent the environment contributes to human exposure, compared to the clinical and veterinary or agricultural domains, needs to be further quantified [17].

Import and spread of resistance by travel

In addition to animals and the environment, the human microbiome, especially the microbiome present in the human gut, also contains resistance genes, albeit at relatively low densities; the fraction of resistance genes among all genes in the gut is estimated to be approximately 0.002 [29]. Thus, by human travel, pools of antibiotic resistance genes are also traveling. According to the World Tourism Organization (UNWTO), international tourist arrivals have increased from 25 million globally in 1950 to 278 million in 1980, 674 million in 2000, and 1,235 million in 2016 [30]. These numbers do not include business and other travel. Even at the estimated low densities of resistance genes in the indigenous human microbiome, international travel at such scale entails the movement and shedding (primarily by defaecation) of huge numbers of resistance genes from one country to another. In addition to the role of (inappropriate) antimicrobial use in humans and animals, the increase of international travel may, thus, also contribute to the worldwide emergence and dissemination of antibiotic resistance since it allows resistant clones or MGEs to be rapidly transported between regions [31]. With multidrug-resistant Enterobacteriaceae being highly prevalent in the community in many parts of the world, especially in Asia [11], Africa [32] and South America [33], there is a huge potential of acquiring and transporting bacteria and/or MGEs, for example from high to low endemic countries, such as the Netherlands, by international or intercontinental travel.
Emerging resistance mechanisms in Enterobacteriaceae

Extended-spectrum β-lactamases (ESBLs)

β-lactams are a group of antibiotics that are among the most commonly used due to their range of spectra (both narrow- and broad-spectrum β-lactams are available) and because of their generally excellent tolerability [34]. β-lactamases are bacterial enzymes that degrade β-lactam antibiotics by hydrolysing the β-lactam ring, thereby abrogating the binding of the antibiotic to their targets, penicillin-binding proteins in the cell wall of the bacteria. β-lactamases are the major cause of bacterial resistance to β-lactam antibiotics and have been the subject of extensive microbiological, biochemical, and genetic investigations [35]. Different classes and types of β-lactamases exist with narrow-spectrum β-lactamases inactivating narrow spectrum β-lactam antibiotics, such as penicillins, and broad-spectrum β-lactamases that can degrade a wider range, including the broad-spectrum β-lactam antibiotics (Table 1).
Extended-spectrum β-lactamases (ESBLs) are, as the name implies, broad-spectrum β-lactamases and have penicillins, narrow- and broad-spectrum cephalosporins and monobactams as substrates. They confer resistance to cephalosporins, such as cefotaxime, ceftazidime and other broad-spectrum cephalosporins and to monobactams, such as aztreonam, but have no detectable activity against cefamycins (e.g. cefoxitin and cefotetan) and carbapenems [36]. ESBLs are most commonly found among isolates of *E. coli*, *Klebsiella pneumoniae* and other Enterobacteriaceae. Common infections with ESBL-producing Enterobacteriaceae (ESBL-E) include urinary tract infections, peritonitis, cholangitis and intra-abdominal abscesses, all of which can lead to bloodstream infections [37]. They may also cause soft tissue-, bone- and respiratory tract infections, especially in patients admitted to health care settings.

Genes that encode for the production of ESBLs are mostly located on MGEs called plasmids. These plasmids and their resistance genes can easily be transferred between Enterobacteriaceae and other bacterial species through horizontal gene transfer. Genes encoding for resistance to other classes of antibiotics, such as trimethoprim-sulfamethoxazole, quinolones and aminoglycosides, are often located on the same plasmids making the isolate multi-drug resistant. For this reason, the selective pressure exerted by a single antibiotic can lead to resistance against multiple other classes of antibiotics [38]. Because often multi-drug resistant, infections that are caused by ESBL-producing bacteria are difficult to treat and often treatment options are limited to carbapenems [37]. ESBL-carrying Enterobacteriaceae have emerged and spread in both hospitals and the community worldwide [39].

**Carbapenemase-producing Enterobacteriaceae (CPE)**

Even more worrisome, Enterobacteriaceae can acquire resistance genes encoding for enzymes called carbapenemases. These carbapenemase-producing Enterobacteriaceae (CPE's) are often designated “extremely drug resistant” (XDR) because they are typically resistant against all commonly used antibiotics. These carbapenemase-encoding genes are often located on plasmids and are active against essentially all β-lactam antibiotics, including the carbapenems, which represent a current last resort class of antibiotics. Infections with CPE are difficult to treat and associated with high mortality [40]. For the treatment of infections with CPE, colistin, tigecycline and fosfomycin, as monotherapy or in combination with carbapenems are used [40, 41]. Especially the polymyxin family of antibiotics, polymyxin B and polymyxin E (colistin) have become the backbone agents in combination regimens which are currently under study [41-44].

Extended-spectrum β-lactamases (ESBLs) are, as the name implies, broad-spectrum β-lactamases and have penicillins, narrow- and broad-spectrum cephalosporins and monobactams as substrates. They confer resistance to cephalosporins, such as cefotaxime, ceftazidime and other broad-spectrum cephalosporins and to monobactams, such as aztreonam, but have no detectable activity against cefamycins (e.g. cefoxitin and cefotetan) and carbapenems [36]. ESBLs are most commonly found among isolates of *E. coli*, *Klebsiella pneumoniae* and other Enterobacteriaceae. Common infections with ESBL-producing Enterobacteriaceae (ESBL-E) include urinary tract infections, peritonitis, cholangitis and intra-abdominal abscesses, all of which can lead to bloodstream infections [37]. They may also cause soft tissue-, bone- and respiratory tract infections, especially in patients admitted to health care settings.

Genes that encode for the production of ESBLs are mostly located on MGEs called plasmids. These plasmids and their resistance genes can easily be transferred between Enterobacteriaceae and other bacterial species through horizontal gene transfer. Genes encoding for resistance to other classes of antibiotics, such as trimethoprim-sulfamethoxazole, quinolones and aminoglycosides, are often located on the same plasmids making the isolate multi-drug resistant. For this reason, the selective pressure exerted by a single antibiotic can lead to resistance against multiple other classes of antibiotics [38]. Because often multi-drug resistant, infections that are caused by ESBL-producing bacteria are difficult to treat and often treatment options are limited to carbapenems [37]. ESBL-carrying Enterobacteriaceae have emerged and spread in both hospitals and the community worldwide [39].

**Carbapenemase-producing Enterobacteriaceae (CPE)**

Even more worrisome, Enterobacteriaceae can acquire resistance genes encoding for enzymes called carbapenemases. These carbapenemase-producing Enterobacteriaceae (CPE's) are often designated “extremely drug resistant” (XDR) because they are typically resistant against all commonly used antibiotics. These carbapenemase-encoding genes are often located on plasmids and are active against essentially all β-lactam antibiotics, including the carbapenems, which represent a current last resort class of antibiotics. Infections with CPE are difficult to treat and associated with high mortality [40]. For the treatment of infections with CPE, colistin, tigecycline and fosfomycin, as monotherapy or in combination with carbapenems are used [40, 41]. Especially the polymyxin family of antibiotics, polymyxin B and polymyxin E (colistin) have become the backbone agents in combination regimens which are currently under study [41-44].
Colistin-resistant Enterobacteriaceae and the mcr-1 gene

As noted above, the emergence of multidrug-resistant Enterobacteriaceae, especially those producing carbapenemases, has reintroduced colistin as a last resort antibiotic for the treatment of severe infections due to Gram-negative bacteria [45]. Except for some intrinsically resistant species, resistance to colistin is relatively rare and long thought to be caused by chromosomal mutations only [46, 47]. However, plasmid-mediated resistance, conferred by the mobilized colistin resistance (mcr-1) gene, was discovered in 2015 in China [48]. Since then, the gene has been described in several bacterial species that were isolated from animals, animal food products, humans and environmental samples from around the world [49-53]. These findings confirmed fears that, plasmid-mediated colistin resistance will spread much faster than the spread of bacterial clones with chromosomally mediated colistin resistance.

Table 1. Examples of common β-lactamases according to the Ambler classification system.

<table>
<thead>
<tr>
<th>Ambler class</th>
<th>Enzyme type</th>
<th>Active site</th>
<th>Characteristics</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Pencillinas / Narrow-spectrum β-lactamases</td>
<td>Serine</td>
<td>Hydrolyse penicillins, inhibited by clavulanic acid</td>
<td>TEM-1, TEM-2, SHV-1</td>
</tr>
<tr>
<td>A</td>
<td>Extended-spectrum β-lactamases (ESBLs)</td>
<td>Serine</td>
<td>Hydrolyse narrow and extended-spectrum β-lactams, including cephalosporins, inhibited by clavulanic acid</td>
<td>SHV-2, CTX-M-like, PER-1, VEB-1</td>
</tr>
<tr>
<td>A</td>
<td>Carbapenemases</td>
<td>Serine</td>
<td>Hydrolyse all β-lactams including monobactams and carbapenems, inhibited by clavulanic acid</td>
<td>KPC, IMI, SME</td>
</tr>
<tr>
<td>B</td>
<td>Metallo-β-lactamases</td>
<td>Zinc</td>
<td>Hydrolyse oxyimino β-lactams, cephamycins and carbapenems, but not monobactams, not inhibited by clavulanic acid</td>
<td>VIM, IMP, NDM, GIM, SPM, SIM</td>
</tr>
<tr>
<td>C</td>
<td>Cephalosporinases</td>
<td>Serine</td>
<td>Hydrolyse cephapemycins, and some oxyimino β-lactams: chromosomally and plasmid mediated, not inhibited by clavulanic acid</td>
<td>AmpC, ACT, CMY, MIR</td>
</tr>
<tr>
<td>D</td>
<td>Oxacillinases</td>
<td>Serine</td>
<td>Hydrolyse oxacillin, oxyimino β-lactams and sometimes carbapenems, variably inhibited by clavulanic acid</td>
<td>OXA, including OXA-4B-like</td>
</tr>
</tbody>
</table>

Adapted from Liscio [54] and Toussaint [55]

Colistin-resistant Enterobacteriaceae and the mcr-1 gene

As noted above, the emergence of multidrug-resistant Enterobacteriaceae, especially those producing carbapenemases, has reintroduced colistin as a last resort antibiotic for the treatment of severe infections due to Gram-negative bacteria [45]. Except for some intrinsically resistant species, resistance to colistin is relatively rare and long thought to be caused by chromosomal mutations only [46, 47]. However, plasmid-mediated resistance, conferred by the mobilized colistin resistance (mcr-1) gene, was discovered in 2015 in China [48]. Since then, the gene has been described in several bacterial species that were isolated from animals, animal food products, humans and environmental samples from around the world [49-53]. These findings confirmed fears that, plasmid-mediated colistin resistance will spread much faster than the spread of bacterial clones with chromosomally mediated colistin resistance.

Table 1. Examples of common β-lactamases according to the Ambler classification system.

<table>
<thead>
<tr>
<th>Ambler class</th>
<th>Enzyme type</th>
<th>Active site</th>
<th>Characteristics</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Pencillinas / Narrow-spectrum β-lactamases</td>
<td>Serine</td>
<td>Hydrolyse penicillins, inhibited by clavulanic acid</td>
<td>TEM-1, TEM-2, SHV-1</td>
</tr>
<tr>
<td>A</td>
<td>Extended-spectrum β-lactamases (ESBLs)</td>
<td>Serine</td>
<td>Hydrolyse narrow and extended-spectrum β-lactams, including cephalosporins, inhibited by clavulanic acid</td>
<td>SHV-2, CTX-M-like, PER-1, VEB-1</td>
</tr>
<tr>
<td>A</td>
<td>Carbapenemases</td>
<td>Serine</td>
<td>Hydrolyse all β-lactams including monobactams and carbapenems, inhibited by clavulanic acid</td>
<td>KPC, IMI, SME</td>
</tr>
<tr>
<td>B</td>
<td>Metallo-β-lactamases</td>
<td>Zinc</td>
<td>Hydrolyse oxyimino β-lactams, cephamycins and carbapenems, but not monobactams, not inhibited by clavulanic acid</td>
<td>VIM, IMP, NDM, GIM, SPM, SIM</td>
</tr>
<tr>
<td>C</td>
<td>Cephalosporinases</td>
<td>Serine</td>
<td>Hydrolyse cephapemycins, and some oxyimino β-lactams: chromosomally and plasmid mediated, not inhibited by clavulanic acid</td>
<td>AmpC, ACT, CMY, MIR</td>
</tr>
<tr>
<td>D</td>
<td>Oxacillinases</td>
<td>Serine</td>
<td>Hydrolyse oxacillin, oxyimino β-lactams and sometimes carbapenems, variably inhibited by clavulanic acid</td>
<td>OXA, including OXA-4B-like</td>
</tr>
</tbody>
</table>

Adapted from Liscio [54] and Toussaint [55]
Implications for public health, clinical care and individual travellers

Insight in the acquisition rate of resistant Enterobacteriaceae by healthy travellers and subsequent transmission to household members will provide better insights in the burden of the influx of resistant bacteria due to international travel in the Netherlands, as well as the potential consequences for infection prevention and clinical management of infections in individuals who have recently returned from travel.

The choice of empirical antibiotic treatment in patients with severe infections (e.g. sepsis) may need adjustment in case of recent travel to certain countries or regions of the world that are associated with increased risk of acquisition and subsequent carriage of resistant bacteria. Duration of carriage will determine how long after their return travellers should be considered at risk of infection with resistant bacteria. Identification of predictors of acquisition may help identify those at highest risk of carriage and infection with multidrug resistant bacteria. This would help to restrict empirically prescribed broad-spectrum antibiotics that cover for such resistant bacteria (e.g. carbapenems) for those who need it, thereby preventing further resistance development without jeopardizing individual health.

Special precautions, including selective screening on admission and temporary isolation of travellers who had been in contact with health care during travel, are successfully applied in the Netherlands. Such precautions may need to be expanded to those who have not been exposed to health care to prevent introduction and spread of antimicrobial resistance into our health care system. When determinants of acquisition and sustained carriage of resistant Enterobacteriaceae are identified, infection prevention guidelines may thus be adjusted to prevent spread of these bacteria in health care facilities in the Netherlands.

In addition to optimizing empirical treatment of infections in travellers seeking medical help after travel, the identification of travel-associated risk factors is needed to identify possible interventions to prevent acquisition of resistant bacteria during travel. Reducing risk as well as increasing possible protective factors are potentially useful, although future research will be needed to study the effect of these interventions on acquisition and sustained carriage of resistant bacteria.

The choice of empirical antibiotic treatment in patients with severe infections (e.g. sepsis) may need adjustment in case of recent travel to certain countries or regions of the world that are associated with increased risk of acquisition and subsequent carriage of resistant bacteria. Duration of carriage will determine how long after their return travellers should be considered at risk of infection with resistant bacteria. Identification of predictors of acquisition may help identify those at highest risk of carriage and infection with multidrug resistant bacteria. This would help to restrict empirically prescribed broad-spectrum antibiotics that cover for such resistant bacteria (e.g. carbapenems) for those who need it, thereby preventing further resistance development without jeopardizing individual health.

Special precautions, including selective screening on admission and temporary isolation of travellers who had been in contact with health care during travel, are successfully applied in the Netherlands. Such precautions may need to be expanded to those who have not been exposed to health care to prevent introduction and spread of antimicrobial resistance into our health care system. When determinants of acquisition and sustained carriage of resistant Enterobacteriaceae are identified, infection prevention guidelines may thus be adjusted to prevent spread of these bacteria in health care facilities in the Netherlands.

In addition to optimizing empirical treatment of infections in travellers seeking medical help after travel, the identification of travel-associated risk factors is needed to identify possible interventions to prevent acquisition of resistant bacteria during travel. Reducing risk as well as increasing possible protective factors are potentially useful, although future research will be needed to study the effect of these interventions on acquisition and sustained carriage of resistant bacteria.
Aims and outline of the studies presented in this thesis

Resistance and travel

As indicated in the previous section, the emergence of antibiotic resistance is threatening our ability to treat common infections. To determine which intervention strategies are most effective in combating antibiotic resistance, insight in the relative contributions of humans, animals, the food chain and the environment to the emergence and spread of antibiotic resistance are needed. Knowledge on the extent to which travellers contribute to antibiotic resistance in the Netherlands and on who is at risk for the acquisition, import and spread of resistant bacteria, is essential in order to prevent and manage this influx and potential subsequent spread in the Netherlands.

Previously published, small to medium sized, prospective cohort studies showed high acquisition rates of ESBL-E among travellers, especially among those who had returned from Southern Asia and Northern Africa. Conflicting results on predictive factors such as antibiotic use and traveller’s diarrhoea were found and duration of ESBL-E carriage and onward transmission within households was insufficiently addressed [56]. Therefore, important knowledge gaps include (1) identification of travel-associated predictors for acquisition and sustained carriage, (2) duration of colonization with resistant strains acquired during travel and (3) probability and dynamics of subsequent transmission of these strains within households.

To address these questions, a large prospective cohort study was performed in 2,001 Dutch travellers and 215 of their non-travelling household members to investigate the acquisition of extended-spectrum β-lactamase-producing (ESBL-E) and carbapenemase-producing Enterobacteriaceae (CPE) during international travel, with a focus on acquisition rates and predictive factors for acquisition, duration of colonisation, and probability of onward transmission. The rationale behind this Carriage Of Multiresistant Bacteria After Travel (COMBAT) prospective cohort study and its methodology and design are discussed in Chapter 2. The results of this study in relation to ESBL-carrying Enterobacteriaceae are presented in Chapter 3, while Chapter 4 focuses on the acquisition and potential onward transmission of CPE in our cohort of travellers. Following the discovery of plasmid-mediated colistin resistance encoded by the mcr-1 gene in 2015, we investigated its presence in bacterial isolates from our cohort of travellers (Chapter 5). This study was followed by detailed investigations to study the global population structure of E. coli and its genetic MGEs carrying the mcr-1 gene using whole-genome sequencing and MLST profiling data from our travel-acquired isolates and those from publicly available databases and the literature (Chapter 6).

Resistance and travel

As indicated in the previous section, the emergence of antibiotic resistance is threatening our ability to treat common infections. To determine which intervention strategies are most effective in combating antibiotic resistance, insight in the relative contributions of humans, animals, the food chain and the environment to the emergence and spread of antibiotic resistance are needed. Knowledge on the extent to which travellers contribute to antibiotic resistance in the Netherlands and on who is at risk for the acquisition, import and spread of resistant bacteria, is essential in order to prevent and manage this influx and potential subsequent spread in the Netherlands.

Previously published, small to medium sized, prospective cohort studies showed high acquisition rates of ESBL-E among travellers, especially among those who had returned from Southern Asia and Northern Africa. Conflicting results on predictive factors such as antibiotic use and traveller’s diarrhoea were found and duration of ESBL-E carriage and onward transmission within households was insufficiently addressed [56]. Therefore, important knowledge gaps include (1) identification of travel-associated predictors for acquisition and sustained carriage, (2) duration of colonization with resistant strains acquired during travel and (3) probability and dynamics of subsequent transmission of these strains within households.

To address these questions, a large prospective cohort study was performed in 2,001 Dutch travellers and 215 of their non-travelling household members to investigate the acquisition of extended-spectrum β-lactamase-producing (ESBL-E) and carbapenemase-producing Enterobacteriaceae (CPE) during international travel, with a focus on acquisition rates and predictive factors for acquisition, duration of colonisation, and probability of onward transmission. The rationale behind this Carriage Of Multiresistant Bacteria After Travel (COMBAT) prospective cohort study and its methodology and design are discussed in Chapter 2. The results of this study in relation to ESBL-carrying Enterobacteriaceae are presented in Chapter 3, while Chapter 4 focuses on the acquisition and potential onward transmission of CPE in our cohort of travellers. Following the discovery of plasmid-mediated colistin resistance encoded by the mcr-1 gene in 2015, we investigated its presence in bacterial isolates from our cohort of travellers (Chapter 5). This study was followed by detailed investigations to study the global population structure of E. coli and its genetic MGEs carrying the mcr-1 gene using whole-genome sequencing and MLST profiling data from our travel-acquired isolates and those from publicly available databases and the literature (Chapter 6).
In addition to our primary objective of studying acquisition of ESBL-E and CPE during travel, we studied the acquisition diarrhoeagenic bacteria, viruses and parasites. The rationale behind these studies is discussed below.

**Diarrhoeagenic bacteria, viruses and parasites and travel**

Limited prospective data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by healthy individuals during travel. In addition to the epidemiological relevance, such data may help to understand the clinical relevance of detected pathogens in the era of extremely sensitive diagnostic testing with real-time PCR (RT-PCR). This could be particularly useful knowledge in the clinical management of travellers who return with (persistent) traveller’s diarrhoea. In Chapter 7, we therefore determined the frequency of travel-associated acquisition of 19 pathogens, as detected by RT-PCR, in a selection of travellers from our cohort.

*Blastocystis* spp. are frequently found in the human gut microflora but their pathogenicity has been under debate for a long time. Inspired by observations from Chapter 7, showing high prevalence before and after travel, additional studies on the geographical distribution and dynamics of acquisition and loss of *Blastocystis* were performed, as described in Chapter 8.

In Chapter 9, the main findings of this thesis and future directions are discussed.
References

GENERAL INTRODUCTION

1.79-87.
