# General discussion and summary

## INTRODUCTION

Healthy travellers can pick up micro-organisms carrying antimicrobial resistance genes when visiting regions of the world where antimicrobial resistance among common pathogens has reached high endemic levels, and import these to their home countries. The microbiota of healthy travellers may, thus, become reservoirs of resistant strains and their antimicrobial resistance genes. However, because acquisition of antimicrobial resistant pathogens is only noticed in travellers when they cause an infection, little is known about carriage of these resistant pathogens by healthy travellers. As it is estimated that the number of international tourists to endemic regions in Asia and Africa will continue to grow at the rate of 4.4% a year (1), it is prudent to investigate the risks of introduction and possible spread of antimicrobial resistance (in particular antibiotic-resistant Enterobacteriaceae) by healthy travellers from the Netherlands, a country known for its restricted antibiotic use and correspondingly low levels of resistant pathogens in its community and health care system.

## MAIN FINDINGS AND DISCUSSION

### Impact of international travel on the endemicity of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) in the Netherlands

Previous small to medium sized studies of international travellers had reported high acquisition rates of ESBL-E, with observed frequencies varying between 21-51% **(Chapter 2)**. To more definitively assess the impact of international travel on carriage of antimicrobial resistant pathogens we investigated the acquisition, persistence of carriage and transmission of ESBL-E by healthy travellers prospectively enrolled in a large, multicenter longitudinal cohort. We found that overall, 34.3% (633/1847) of travellers acquired ESBL-E during their international travel **(Chapter 4)**. The median duration of carriage after travel was 30 days and approximately 11% remained a carrier of ESBL-E for more than one year after travel. Also, transmission to close household contacts was observed. Acquisition rates varied per subregion from 5.9% in travellers returning from Northern America/ Europe/Oceania to 75.1% in travellers returning from Southern Asia. Moreover, the frequency of acquisition also varied widely per country within these regions.

We determined the contribution of the import of ESBL-E by healthy travellers on a population level by using the acquisition rates we found per subregion in our study and by taking into account the total number of Dutch travellers visiting these subregions annually (2). From these calculations we estimate that each year between 3.0% and 7.1% of the Dutch population acquire an ESBL-E during travel to Asia, Africa and Latin-America.

In absolute numbers that is a yearly influx of 500.000 to 1.2 million Dutch travellers with ESBL-E. So far no other factor in the Netherlands has been identified accounting for so much ESBL-E influx in to the Netherlands. According the Central Bureau for Statistics of the Netherlands the two most popular regions visited for long holidays are Western Asia and Northern Africa. The large number of visitors to Western Asia can be explained by the preference of Dutch tourists for all-inclusive hotels in Turkey (personal data Travel Clinic Harbor Hospital), and by first and second generation immigrant inhabitants with Turkey as their migration background, as this is the largest group in the Netherlands with a non-western migration background. The large number of visitors to Northern Africa can be explained by the preference of Dutch tourists for all-inclusive hotels in Egypt (personal data Travel Clinic Harbor Hospital) and by first and second generation immigrant inhabitants of the Netherlands that have Morocco as their migration background, as this is the second largest group in the Netherlands with a non-western migration background. Our study found high acquisition rates (42.0-42.9 %) by travellers to these two regions. With our calculations we estimate that, Dutch travellers to Western Asia and Northern Africa together account for an annual influx of as much as 320.000 to 870.000 travellers importing ESBL-E into the Netherlands. Thus, although travellers to Southern Asia have the highest risk (75.1%) to acquire ESBL-E, travellers to Western Asia and Northern Africa contribute most to ESBL-E endemicity at the level of the Dutch population. Since the Dutch population has a long history in international travel and global trade, including visits to regions with high endemic levels of antimicrobial resistance, the yearly influx of ESBL-E yielded by healthy travellers is a major determinant of the ESBL-E endemic level in the Netherlands.

A limitation of our study is that our study population included mostly native Dutch travellers who came to the travel clinics for vaccinations and travel advice. By using data of the Central Bureau of Statistics of long holidays among the Dutch population, we were able to include the Dutch inhabitants of Turkish or Moroccan origin who go on holiday to their country of origin in our calculation of ESBL-E influx by Dutch travellers contributing to ESBL-E endemicity in the Netherlands. We were not able to take into account the contribution to ESBL-E endemicity in the Netherlands of recently arrived refugees. In 2018 there were almost 26.000 asylum requests in the Netherlands most of which were made by Syrian refugees (3). Research in Germany showed that 35% of Syrian refugees were carrier of ESBL-E. Since the number of refugees is much lower than that of Dutch inhabitants travelling internationally, the contribution of refugees is expected to be much smaller than that of Dutch travellers (4).

Another limitation of our study is that we were not able to include travellers to countries within Europe or other countries for which no vaccinations or travel advice is needed. Data show ESBL prevalence rates among hospital *E. coli* isolates in the range of 10-25% for popular summer holiday destinations including Spain, Portugal, Greece

and Israel (5-7). As resistance genes can be acquired in the first days after arrival in high endemic countries (John Penders, not published), also short holidays to these popular destination within Europe are likely to contribute to the ESBL-E endemic level in the Netherlands.

Although little more than half of travellers who acquired ESBL-E during their travel lost their ESBL-E within one month after return, 11.3% of travellers remained a carrier of ESBL-E 12 months after return. These sustained carriers also contribute to the ESBL-E endemic level in the Netherlands, since 11.3% of 500.000 to 1.2 million healthy travellers will yield 56.000 to 135.000 long term carriers with imported ESBL-E carriage up to twelve months after return. Our review showed that other traveller studies found varied duration of carriage rates from 5% to 24% at 6 months after return. Our Cox regression analysis showed travellers who carried *E. coli* (vs *K. pneumoniae*) or an ESBL-gene from CTX-M group 9 (vs CTX-M group 1) were at increased risk for sustained carriage. These results suggest plasmids carrying CTX-M group 9 provide a colonization advantage over plasmids carrying CTX-M group 1. CTX-M group 9 ESBL genes are known to be dominant in China. In line with this is the observation that travellers to Central- and Eastern Asia in our study cohort were at increased risk for acquisition of CTX-M group 9 (OR 3.3, $CI_{95}$ 1.4-7.5) and ESBL-gene CTX-M-14/18 (OR 3.5, $CI_{95}$ 1.5-7.9), when compared to travellers who did not visit this subregion **(Chapter 5)**. Although our Cox regression analysis did not demonstrate that travel to Central and Eastern Asia posed an increased risk for sustained carriage, our data do suggest that these travellers are more at risk for sustained carriage of ESBL-E, because of the greater chance to acquire ESBL CTX-M group 9 when compared to travellers to other destinations. Another study found travellers returning from Asia had significantly longer sustained carriage compared to travellers from sub-Saharan Africa or Latin America (8). Other studies also found CTX-M group 9 to be associated with sustained carriage in travellers returning from Asia, and in patients from hospital, long-term care facilities and primary care practices (9, 10). A recent study which investigated long-term ESBL-E carriage in the adult Dutch community suggested that in half of persons who continued to carry CTX-M group 9 ESBL genes for up to 8 months, persistence of successful clones rather than horizontal gene transfer was explanatory for the persistence of these ESBL-E. They found that those with antibiotic use during the last 6 months, proton pump inhibitor use or living within 1,000 meters of a pig farm are at increased risk for sustained ESBL-E carriage (11).

With our mathematical model which took factors into account like actual total household size, information which was derived from our questionnaires, we estimated that there was a 12% probability of transmission from ESBL positive persons to ESBL negative persons in households. Perhaps an epidemiologically more popular way to describe transmission rates is the basic reproductive ratio ($R_0$), which is the total number of secondary ESBL-E cases generated from the total number of ESBL index persons introduced

into a susceptible population of household contacts. A basic reproductive ratio >1 is considered as the threshold for ESBL-E to spread in the susceptible population (12). In our mathematical model of onward transmission of ESBL-E in households of travellers, we estimated person-to-person transmission to occur at a rate of 0.0013 per colonized person a day and the average duration of carriage to be 100 days. $R_0$ is calculated to be 0.20, by multiplying the transmission rate (0.0013/day) with the average duration of carriage (100 days) and the average number of household numbers (1.54). As this $R_0$ of 0.20 is far below 1.0, we conclude that transmission to household members does not lead to significant spread within the susceptible population. To put our finding in perspective, a higher $R_0$ of 0.90 was found for MRSA transmission from patients to household contacts (13). Another Dutch study using the same mathemathical model as ours for onward transmission of ESBL-E in households of recently discharged patients, estimated a similar average duration of carriage of 111 days. However, they estimated a higher person-to-person transmission rate of 0.0053 per colonized person a day, which is most likely explained by the more frequent and longer exposure times between caregiving household members to discharged patients (14). Although transmission of ESBL-E in households of travellers unlikely leads to sustained spread, a transmission rate of 12% does contribute to the endemic level of ESBL-E in the Netherlands, as 12% of 500.000 to 1.2 million healthy travellers implies 60.000 to 144.000 travellers who transmit ESBL-E to their household members yearly.

**Travel-associated risk factors**

Travel destination is the dominant determinant for acquisition of ESBL-E in our cohort. Our travellers to Asia had a higher frequency of ESBL-E acquisition, which is probably due to the widely spread distribution of ESBL-E in these regions and, therefore, a high risk of food-contamination. In our overall analysis, occasional food consumption from street vendors was associated with an increased risk for ESBL-E acquisition, whereby this risk further increased when food from street vendors was consumed daily. As diet-associated predictors might differ per subregion, we performed a stratified analysis per subregion, which identified daily food consumption at a hostel or guesthouse in southern Asia and consumption of raw vegetables in southeastern Asia as predictive factors. The highest ESBL-E acquisition rate of 88.6% was found in travellers returning from India. Apart from the overuse of antibiotics in humans and animals, the high level of ESBL-E endemicity in India can, at least in part, be explained by its lack of access to proper sanitation facilities, with less than 40% of its population having access to sanitation (15). Also, several large and small pharmaceutical factories producing antibiotics are located in India. Research in the vicinity of those factories have shown that tons of antibiotics such as ciprofloxacin are released in the environment every year (16). In such setting, the selective pressure of just one antibiotic likely leads to the co-selection of multi-drug resistant isolates and

drive their spread (17). One study demonstrated that the use of fluoroquinolones during travel selected for ESBL-E acquisition during travel (18). Moreover, a recent meta-analysis showed 12.4% of antibiotics were substandard or falsified in low,- and middle-income countries, which is also thought to contribute to increasing antimicrobial resistance in these countries (19).

In our multivariable analysis we found that antibiotic use (in particular the use of quinolones) during travel, traveller's diarrhoea, and pre-existent chronic bowel disease were independently associated with an increased risk of ESBL-E acquisition. These conditions are all known to be associated with a dysbiosis of the human gut microbiota (20-22). Therefore, dysbiosis of the human microbiota with subsequent decreased colonization resistance might be a biologically plausible common precondition or mechanism for increased susceptibility to the acquisition of ESBL producing micro-organisms. Dutch national guidelines for the use of antibiotics advise the prescription of ciprofloxacin or azithromycin for moderately severe traveller's diarrhoea in patients with chronic conditions like diabetes mellitus or use of immunosuppressive drugs. Both ciprofloxacin and azithromycin are known to induce a dysbiosis of the human microbiota (20, 23, 24). The traveller study of Kantele *et al.* demonstrated ESBL-E acquisition rates in travellers to South-East Asia without traveller's diarrhoea and without antibiotic use, in those with traveller's diarrhoea but without antibiotic use, and in those with traveller's diarrhoea and antibiotic use to be 19%, 32%, and 69%, respectively. For travellers to South Asia similar rates were reported, i.e. 23%, 47% and 80%, respectively. These results suggest that the resistance of travellers to become colonized by ESBL-E is synergistically decreased by diarrhoea in combination with the use of antibiotics (25, 26). In our study we found travel-related and non-travel related risk factors for ESBL-E acquisition, all known to be associated with gut dysbiosis. However, the effect of travel itself on the composition and function of the human gut microbiota remains unknown, as only a single study has examined the gut microbiota of diarrhoea-free travellers. This study found the gut microbiome in healthy, diarrhoea-free, travellers to be dysbiotic, suggesting that travel itself could be associated with temporary dysbiosis of the gut microbiota. However, this was merely an assumption or hypothesis, since pre-travel faecal samples of these healthy travellers were not analysed (27).

### ESBL-E carriage in the community

In **chapter 5** we investigated ESBL prevalence and associated risk factors in travellers prior to their index travel. These results are, to some extent, reflective for ESBL-E carriage and predictors in the Dutch general adult population. We found a prevalence for ESBL-E carriage of 6.1% (136/2216) prior to travel, which was slightly higher (versus 4.5% and 5.1%) and lower (versus 8.6%) compared to previous Dutch studies among healthy individuals (28-30). From the multivariable risk factor analysis, previous antibiotic use in

the past three months and previous travel outside of Europe in the past twelve months were the major risk factors for ESBL-E carriage prior to travel.

The association between carriage of ESBL-E prior to travel and recent antibiotic use found in this study, may reflect reduced colonization resistance to ESBL-E acquisition from external sources as well as selection of ESBL-E present in the gut prior to exposure, albeit at densities below the detection threshold of routine microbiological culture. Especially the use of beta-lactam antibiotics was associated with ESBL-E carriage prior to travel. Beta-lactams are the class of antibiotics most commonly prescribed by general practitioners, by doctors from outpatient clinics and by dentists in the Netherlands (31). Quinolones are less often prescribed, which may help to explain why we did not find a significant association between its use and ESBL-E carriage prior to travel. In the Netherlands the use of amoxicillin and amoxicillin clavulanic acid in outpatients has been declining over the past decade (31). Future research has to determine whether, and if so, to what extent, this decline in the use of beta-lactam antibiotics will be accompanied by a decrease in ESBL-E carriage in the community.

The association between carriage of ESBL-E prior to travel and travel outside of Europe was particularly strong for travel to Asia (Eastern Asia), Africa (Northern Africa) and Oceania (Australia and New Zealand). Other studies on ESBL-E carriage in the community of countries with prudent use of antibiotics also found that those who travelled to Asia and Africa in the past twelve months are at increased risk for ESBL carriage (29, 30, 32, 33). The finding that travel outside of Europa is a major risk factor for ESBL-E carriage prior to travel confirms our finding that travellers returning from high endemic regions for ESBL-E substantially contribute to the ESBL-E endemicity in their country of origin. Moreover, in our population of travellers, the population attributable risk was greatest for travel outside of Europe; it's attributable risk was two times higher than that for antibiotic use. In our cohort of travellers the prevalences of CTX-M-15 and CTX-M-14/18 prior to travel were 31.3% (40/128) and 21.9% (28/128), respectively. Previous other Dutch studies found lower or similar proportions (13-19%) of *bla*CTX-M-14 in the community. Although our study did not have the statistical power to investigate if CTX-M-14 carriage prior to travel was associated with previous travel to CTX-M 14 endemic regions, it is probable that these CTX-M-14 genes were acquired during previous travel to CTX-M-14 endemic regions. Since CTX-M-14 is not prevalent in the Netherlands it is less likely that CTX-M-14 carrying Enterobacteriaceae were acquired in the Netherlands (29, 30, 34). With the unabated growth in international travel by tourists, business travel, but also by migrants and refugees, it is expected that the impact of international travel on ESBL-E carriage and infection in the community will keep growing the coming years.

We found that participants working in healthcare with daily patient contact tended to be at increased risk for ESBL-E carriage prior to travel, although the association was not statistically significant. The number of participants working in healthcare may have

been too low to reach statistical significance. Just a few studies reported ESBL-E carriage rates among healthcare workers, which ranged from 3 to 21% (35-37).

We did not find an association between consumption of chicken meat or other food products and ESBL-E carriage prior to travel. In accordance with our findings, none of the studies investigating potential predictors for ESBL-E carriage in countries with prudent antibiotic use did identify meat products as a predictor for ESBL-E carriage, except for one study (38). Recent studies using whole genome sequencing show that there is no evidence of clonal spread of ESBL *E. coli* through the food chain, rather they suggest that distinct plasmids contribute to the spread of antibiotic resistance between different reservoirs (39). However, a recent study failed to demonstrate a epidemiological link of ESBL genes and plasmid types between isolates from livestock or food reservoirs and human isolates from the community (40).

We also did not find the use of antacids as a risk factor for ESBL-E carriage in our study population before travel. In addition, antacid use during travel was also not identified as a risk factor for ESBL-E acquisition during travel. The use of prescribed antacids has been identified as risk factor for ESBL-E carriage in the Dutch population, and the use of proton pump inhibitors, the dominant class of antacids prescribed here, has been identified as a risk factor for ESBL-E carriage at hospital admission (29, 41). Moreover, the use of proton pump inhibitors was recently reported to be associated with sustained carriage of ESBL-E in the Dutch community (11). Proton pump inhibitors have been associated with a dysbiosis of the gut microbiota (42-44). We did not make a distinction between proton pump inhibitors (which are prescribed antacids) and neutralizing antacids (that can be obtained over the counter medicine without prescription). Thus, it could be that our participants were mostly using neutralizing antacids, which have so far not been associated with ESBL-E carriage.

As we have shown that returning international travellers are a dominant source for influx of ESBL-E (mostly ESBL-*E.coli* ) in the Netherlands, contributing to ESBL-E endemicity in the community, it is assumed that travellers also contribute to the prevalence of community and hospital acquired infections caused by ESBL-E. A recent Dutch study provided evidence that ESBL *E. coli* strains causing hospital infections most likely originated from the community. These authors analysed trends of ESBL *E. coli* and *K. pneumoniae* infections among patients attending a general practitioner and in hospital patients from 2008-2012. They found an increase in the proportion of ESBL among *E. coli* isolates from urine and blood samples in both general practitioner patients and hospital patients, including ICU patients. However, the proportion of ESBL among *K. pneumoniae* isolates from urine and blood samples did not increase in hospital patients, including ICU patients. Therefore, the rise of ESBL-*E. coli* in the hospital is most likely explained by increased influx from the community, as *E. coli* is a common community pathogen and, therefore, little affected by infection prevention policies in hospitals. In contrast,

the prevalence of ESBL- *K. pneumoniae* in the hospital did not increase, suggesting that infection prevention policies restricted the spread of this hospital pathogen (45). However, from 2013 to 2018 the percentages of both ESBL- *E. coli* and *K.pneumoniae* have risen to 2-6% for *E. coli* and 4-9% for *K. pneumoniae*, with the lowest percentages in GP patients/outpatients and the highest percentages in inpatients/ ICU patients (31). The reasons behind the overall rise of ESBL- *K. pneumoniae* need to be further investigated. Interestingly, 19% of bean sprouts samples in the Netherlands were found to be contaminated with mostly ESBL-*K.pneumoniae* in the absence of ESBL-*E. coli* (46).

**Implications of our findings: who is at risk for carriage of antibiotic resistant Enterobacteriaceae**

To control MRSA in hospital, current standard care, at least in many Western countries including the Netherlands, include risk assesments for MRSA carriage by questionnaires to be taken for every patient at the time of their admission. When patients have one or more risk factors for MRSA carriage they are isolated and first screened for MRSA. For carriage of ESBL-E and carbapenemase producing Enterobacteriaceae (CPE), hospitalization abroad, especially if treated on the ICU, is likewise associated with an increased risk (4). Moreover, infections with CPE in countries with very low CPE prevalence have been associated with prior hospitalization in CPE endemic regions (47). Currently, to control antibiotic resistant Enterobacteriaceae, patients are asked whether they have been examined, treated or admitted to a hospital abroad in the preceding two months, or if longer than two months ago whether they have been operated in a hospital abroad or have wounds. If so they are screened for antibiotic resistant Enterobacteriaceae and other (multidrug) resistant bacteria. However, this approach does not encompass the latest insights into the risk for ESBL-E carriage associated with recent international travel. Several recent studies, including our large cohort study, clearly identified travel to certain regions in Asia and Africa to be associated with a high risk for ESBL-E carriage, even without hospitalization abroad. These findings would argue that patients who have recently travelled to ESBL-E endemic regions need to be screened for ESBL-E carriage at the time of admission to hospital. As it is not feasible and effective to screen all patients with recent travel abroad, screening should focus on those patients with a clearly increased risk for ESBL-E. The first question is which regions or countries can be considered as high ESBL-E endemic regions? As reliable surveillance data on ESBL-E carriage rates are lacking for most low to middle income countries in Asia and Africa (48), data on ESBL-E acquisition in returning travellers might be used as a surrogate marker for ESBL-E prevalence in the community in these countries. As acquisition rates vary widely per country within subregions, for example acquisition in South-Eastern Asia varied from 19.0% in Indonesia to 72.2% in Vietnam, it would be prudent to ascertain ESBL-E endemicity per country. If we would define high ESBL-E endemic countries as

those from which >40% of travellers return with ESBL-E, current data suggests that patients returning from India, Egypt, Nepal, Vietnam, Peru, China, Myanmar, Thailand, Sri Lanka, Uganda and Turkey should be screened for ESBL-E on admission to hospital. Unfortunately, this list is not complete since it only includes countries which are popular, intercontinental, travel destinations. This lack of completeness is also illustrated by data showing ESBL prevalence rates among hospital *E. coli* isolates in the range of 25-50% in Italy, Morocco, Cyprus, Slovakia and Bulgaria (5, 49, 50). These high ESBL *E. coli* hospital prevalence rates suggest that the ESBL carriage in the community of these countries is also high, as *E. coli* is a true community pathogen. Therefore, admitted patients recently returning from these countries may also have to be considered for admission screening.

The second question to consider is how long after return should patients be screened on admission to hospitals. Our COMBAT study, which is the largest traveller study so far, revealed that 43%, 25%, 14%, and 11% of travellers with ESBL-E acquisition during travel still carried their ESBL-E at 1, 3, 6, and 12 months after their return, respectively. The second largest traveller study, the VOYAG-R study reported somewhat lower sustained carriage rates of 34%, 10%, 5% and 2% at 1, 3, 6, and 12 months after return, respectively (8). They concluded that ESBL-E acquisition during travel was relatively short-lived afterwards. However, their rates are not comparable with the rates we reported because, for unclear reasons, when calculating carriage rates they included missing samples in the denominator, thus assuming that these would be negative. For example, at 12 months after return only 8 of 227 travellers provided a stool sample, of whom 5/8 (62%) still carried ESBL-E. The stated carriage rate of 2.2% (5/227) 12 months after return is, thus, a gross underestimation as 219 samples were missing. Therefore, we suggest that the sustained carriage rates from the COMBAT study are more accurate. I would, therefore, suggest that patients with travel to ESBL-E high endemic regions (as specified above) in the preceding 1- 3 months, whether they traveled as tourists, migrants or refugees should be screened for ESBL-E at the time of their admission to a hospital, as 25-43% of travel acquired ESBL-E persisted in the gut beyond that timepoint.

Our findings also suggest that patients with recent international travel and antibiotic use during travel or pre-existent chronic bowel disease should be screened for ESBL-E carriage when admitted to the hospital.

In our study, we did not analyse the subsequent risk for infection with ESBL-E in travellers with ESBL-E acquisition during travel. There is not much data on travellers colonized with ESBL-E or CPE and their subsequent risk for developing an infection with these antibiotic resistant Enterobacteriaceae. One traveller study followed 90 travellers with ESBL-E carriage up to one year after travel and reported no laboratory evidence of pyelonephritis or other infections with ESBL-E (25). A French study prospectively screened patients who were admitted to the Infectious Diseases ward and had travelled abroad in the past twelve months. 5/191 travellers had a clinical infection by multidrug

resistant Gram-negative bacteria and 18/191 only carried such strains, of which 22/23 were ESBL-E. Of the five travellers with infection, four had an urinary tract infection with ESBL producing *E. coli*. Infection or carriage with ESBL-E in patients was ten times higher in those who travelled abroad in the past twelve months compared those wo did not travel abroad (51). Moreover, other studies identified international travel as a risk factor for ESBL-E community acquired urinary tract infections more than a decade ago (52-54). Overall, these studies indicate travellers who acquired ESBL-E during travel can develop an infection with the ESBL-E strain imported from abroad.

Specific attention is warranted for patients admitted for transrectal ultrasonography-guided prostate biopsy, elective colorectal surgery and liver transplantation as ESBL-E colonization in patients undergoing these procedures have a higher risk for post procedure ESBL-E infection (55-58). A recent study among patients admitted for elective colorectal surgery showed the risk for deep surgical site infections was doubled in patients who carried ESBL-E versus non-carriers, and the causative pathogen of the surgical site infection was more likely to be ESBL-E versus in non-carriers (57). The higher risk for surgical site infections in patients carrying ESBL-E is most likely explained by the failure of the routinely recommended prophylaxis with cefazolin and metronidazole to cover for ESBL-E wound contamination during intra-abdominal surgery (59). These findings suggest patients admitted for elective colorectal surgery who have recently travelled to high endemic ESBL regions should be screened for carriage of ESBL-E, so that either the prophylaxis regimen is adjusted or adjustment of empirical therapy will be considered, in case surgical site infection follows after the procedure. More research is needed to weigh the (dis)advantages of targeted surgical prophylaxis in ESBL-E carriers.

The high rates of co-resistance to other classes of antibiotics are a further worry. We observed resistance to gentamicin, trimethoprim-sulfamethoxazole, ciprofloxacin and multidrug resistance in 30.8%, 64.4%, 44.8%, 44.3%, respectively, among ESBL *E. coli* strains isolated from returning travellers. Our finding of high co-resistance rates among travel-acquired ESBL *E. coli* strains requires doctors to consider adjusting their empirical therapeutic regimens in patients who recently travelled to a high ESBL-E endemic country. In line with antibiotic stewardship, the risk factors identified for ESBL-E carriage in returning travellers can be used to make a more informed assessment of the personal risk of ESBL-E infection, thereby limiting the prescription of second tier antibiotics to those with an evident increased risk for ESBL-E infection. Recently, risk scoring methods based on case-control studies have been developed to predict bloodstream infection caused by ESBL-E in patients with suspected community sepsis. The so-called Stockholm-score (60) includes healthcare contact abroad within 6 months in addition to a history of prior ESBL-E positive culture or prostate biopsy when predicting ESBL-E community bloodstream infection. If one of these risk factors is present, the authors suggest empirical treatment has to be adjusted. Although the severity of disease and prior antibiotic use

increased the risk for ESBL-E infection, these risk factors were not useful as a tool for guiding empirical therapy as these were present in both cases and controls. However, the severity of illness should be considered in the decision to adjust empirical therapy, with the aim to limit unnecessary use of carbapenems (61).

From a prevention perspective, travellers to endemic areas should be informed about possible exposure to multidrug resistant micro-organisms, especially ESBL-E and CPE, while travelling and how best to limit their risk for ESBL-E acquisition. They should be advised by travel clinics to take the usual hygiene measures to prevent traveller's diarrhoea, to refrain from antibiotic use when diagnosed with self-limiting gastroenteritis, and to avoid eating food from street vendors. These are, so far, the only factors which can be acted upon, as our multivariable risk factor analysis showed that additional actions including regular hand hygiene did not protect against ESBL-E acquisition. Also, we did not find that probiotics had a protective effect on ESBL-E acquisition among travellers. Similarly, a randomized controlled trial showed travellers using probiotics had similar ESBL-E carriage rates compared to the control group of travellers not using probiotics (62).

### Impact of international travel on carbapenemase-producing Enterobacteriaceae (CPE) in the Netherlands

In 2010 the first case of infection with a KPC-2 producing *Klebsiella pneumoniae* was reported in a patient after hospitalization in Greece, who later died of pneumoniae caused by this CPE. In the same year, two patients with carriage of NDM-1 producing *K. pneumoniae* were reported after they had travelled in India (63). The danger of CPE was demonstrated in 2010 when a large outbreak of OXA-48 carbapenemase producing *K. pneumoniae* occurred in the Maasstad Ziekenhuis in Rotterdam, a medium to large sized hospital in the Netherlands. Molecular typing showed the OXA-48 producing *K. pneumoniae* strain to be identical or very closely related to other OXA-48-positive *K. pneumoniae* isolates identified in France and Morocco, suggesting the strain originated from Morocco (64). The prevalence of CPE among clinical isolates in the Netherlands has up to now been low (<1%). Medical microbiologists and health care politicians are very much aware of the danger of CPE for which, an action plan designated "Netherlands CPE green in 2025" was developed in 2015. This is a campaign that aims to keep the Dutch CPE statistics in the "green" zone, i.e. at levels below <1% up to the year 2025.

In our cohort of 2,001 international travellers we found 5 travellers (0.25%) who acquired CPE during their travel **(Chapter 6)**. The CPE genes acquired by these travellers were NDM-1/2, NDM-7, OXA-48, OXA-244 and IMI-2 that were acquired in South-East Asia/Eastern Asia, Myanmar, Turkey/Greece, Indonesia and Myanmar, respectively. Persistence of CPE colonization up to 1 month after return was found in two travellers, and up to 6 months after return in one traveller. Moreover, we found evidence of transmis-

sion of CPE from a traveller who had acquired OXA-244 in Indonesia to his household member, after returning home.

Only three previous studies have reported CPE acquisition in returning travellers who were not hospitalized abroad. These included one French traveller with an OXA-181 producing *E. coli* returning from India, a French traveller and Swiss traveller with NDM-1 producing *E. coli* acquisition from India and an OXA-48 producing *E. coli* acquired by a Dutch traveller returning from Egypt (26, 65, 66). Again, these observations provide evidence that travellers can acquire CPE during travel without being hospitalized abroad.

In countries in Asia with high CPE endemicity, the percentage of CPE infections that have its origin in the community have been reported to be as high as 29.5%. However, in Europe and the United States the percentage of CPE infections that are community acquired can be high as well, with reported proportions up to 18.2% (67).

In low endemic CPE countries, like France, preceding travel to Asia has been identified as a risk factor for CPE infection in hospitalized patients (68).

Although CPE acquisition rates in travellers are low, screening for CPE may be considered as possible spread of CPE can lead to large outbreaks in the hospital. Such admission screening for CPE should take into account the most popular travel destinations of Dutch inhabitants (2, 7) (personal data Travel Clinic Harbor Hospital). High endemic CPE regions in which Dutch travellers are most likely to be exposed to CPE are Turkey and Egypt (OXA-48), Italy (KPC), Spain (OXA-48) and Greece (KPC, VIM) (69-71). However, one should also consider to include India, Pakistan, Bangladesh, and Myanmar in its admission screening, countries which are less frequently visited by Dutch inhabitants, but are highly endemic for NDM-producing Enterobacteriaceae, (72-74).

**Impact of international travel on mcr-1 in the Netherlands**

In 2015, the scientific community was stunned by a report describing the discovery in China of a plasmid based gene, designated *mcr-1*, that encoded for colistin resistance (75). Following this new report, we analysed our collection of ESBL positive samples from returning travellers for *mcr*-1 presence. Six travellers returning from different geographic regions were identified to have acquired the mcr-1 gene, providing clear evidence that the mcr-1 gene had already spread worldwide at the time of enrolment in 2013-2014 of participants in our cohort study **(Chapter 7)**. Indeed, soon after its discovery, many reports described the mcr-1 gene in isolates from animals, animal food products, humans and environmental samples from around the world. In China a hospital outbreak (n=6) of a *mcr-1*-producing *K. pneumoniae* strain among children with acute leukaemia was described (76).

In our review we aimed to analyze the dynamics behind this worldwide spread of *mcr-1* **(Chapter 8)**. By reviewing whole-genome sequences and MLST profiles from 65 published *mcr-1* carrying *E. coli* human, animal and environmental isolates we found

the worldwide dissemination of *mcr-1* is driven mainly by highly promiscuous plasmids rather than *mcr-1*-carrying clones. More regional circulation and dissemination of the *mcr-1*-carrying plasmid IncHI2 was found in Europe and IncI2 in Asia.

Of the 65 *E. coli* isolates, 4 carried carbapenemase encoding genes (NDM-5 from China and USA) and KPC-2 (from Singapore). Moreover, 29/65 (44.6%) carried plasmid-encoded CTX-M ESBL genes, and 10/65 (15.4%) carried plasmid mediated quinolone resistance (qnr) genes. However, the co-resistance rates may not be representative, as there is a significant selection bias of the published *mcr-1* carrying *E. coli* isolates, since most studies searched for *mcr-1* in their existing ESBL-E collections. Interestingly, we found a high percentage of florfenicol (48.9%) and novobiocin (100%) resistance, antibiotics that are only used in food animals, strengthening the current hypothesis that *mcr-1* emerged first in animals.

Although we found the population of *mcr-1*-carrying *E. coli* to be highly diverse, it was dominated by two large groups of genetically related isolates. One of the groups was centered on ST10, which has a high prevalence among the microbiota of humans and food animals and possesses a range of antimicrobial resistance genes (among which plasmid-carried CTX-M ESBL genes) that is more extensive compared to other ST types, suggesting it has an intrinsic propensity for acquiring AMR genes. Therefore, we hypothesized that certain commensal *E. coli* populations, including ST10, may act as a reservoir for the *mcr-1* gene, which likely explains their over-representation in our study.

In the Netherlands a very low prevalence of *mcr-1* was recently reported in only 2/576 (0.35%) fecal samples of patients attending the Leiden University Medical Center, a tertiary hospital in the Netherlands, this gene was detected by real-time PCR. These patients had no history of recent travel nor of recent colistin use (77). Another Dutch study found 2/18 phenotypically colistin resistant *E.coli* isolates, as determined by Vitek susceptibility testing, to harbour the *mcr-1* gene (78). In the meantime, other plasmid mediated genes encoding for colistin resistance have been identified (labeled *mcr-2* to *mcr-8*). The low prevalence of *mcr-1* in combination with a low prevalence of CPE found in the Netherlands, suggests health care in this country is currently not facing a substantial threat of extremely resistant or even pan-drug resistant Gram-negative bacteria in the near future. A low prevalence of *mrc-1* (1.5%) was detected in collections of ESBL *E.coli* cultured from retail chicken meat in the Netherlands in 2009 and 2014 (79), suggesting *mrc-1* reservoirs have been present in Dutch animal husbandry for at least a decade. Also, the rate of *mrc-1* positive *E.coli* isolated from animals (including chicken) coming to slaughter in European countries is low (0.7%) (80). Interestingly, a recent study found a large variation in the prevalence rate of *mcr-1* (2-39%) in retail chicken meat bought from different supermarket chains throughout the Netherlands (81). As the country of origin of the chicken meat could not be traced back, the variation might be explained by the fact that the chicken meat originated from different farms.

In Tunesia, *mcr-1* prevalence in chicken from different farms varied from 20-83% (82). In one of our traveller's *E. coli* isolate acquired in Tunesia, the *mcr-1* carrying plasmid was identified as an INcHI2-type backbone of the ST4 pMLST subtype co-carrying a CTX-M-1 ESBL gene, which was also described in all *mcr-1* carrying plasmids from *E. coli* isolates from chicken farms in Tunesia.This suggests that transmission from chicken meat to the traveller might have occurred, as the traveller reported consumption of beef, chicken and eggs (82).

Since our review revealed *mcr-1* carrying *E. coli* isolates along with carbapenemase encoding genes, one should be aware of the possibility of the emergence of untreatable pandrug resistant Enterobacteriaceae and the possible danger of import of these pandrug resistant Enterobacteriaceae by travellers to their home country or from the veterinary sector of society.

## FUTURE RESEARCH

For the future, first of all it is important to recognize antimicrobial resistance is a problem that affects all regions, therefore a global solution to tackle the problem of antimicrobial resistance is needed. Indeed, the WHO has launched such a program, a global action plan on antimicrobial resistance (48). To be able to solve the problem of antimicrobial resistance at its roots, awareness and political will to address the problem in countries with high rates of antibiotic resistance, including resistant strains belonging to the family of Enterobacteriaceae, is needed (83). The finding that neonatal sepsis caused by ESBL-E is a risk factor for neonatal death in low income countries with high level ESBL-E endemicity, highlights the severity of the problem (84). One of the key actions needed is to gather more surveillance data on antimicrobial resistance, as data on ESBL and CPE prevalence rates are lacking in many low- and middle income countries (49). The implementation of this action plan is underway, and, predictably, meets difficulties since it involves funding issues, public awareness, multisectoral collaboration and political will to combat antimicrobial resistance (85, 86).

Secondly, new antibiotics are needed to combat antimicrobial resistance in gram negative bacteria. If one thing is sure, it is that antimicrobial resistance will pose new challenges for treatment. Development of new antibiotics, like new combinations of already-licensed beta-lactams and beta-lactamase inhibitors, and reappraisal of "old antibiotics" should be encouraged by the government and other stakeholders (87).

Thirdly, more detailed molecular characterization of strains from hospital, community and travellers are needed to better understand the dynamics of import, spread and sustained carriage of ESBL-E by travellers. Available data show that the most frequent ST-types found in *E. coli* from the Dutch community and in a Dutch traveller study were

similar, namely ST131, ST10 and ST38 (11, 29, 88). Another Dutch study in the community found persistent carriage of CTX-M group 9 by ST131 and ST10 *E. coli.* Persistent carriage in this study was most likely explained by successful clones, as CTX-M group 9 was carried by the same ST type *E. coli* and the same plasmid in the first and last positive sample (11).

Fourthly, more insights are needed in the dynamics and functioning of the human microbiota in relation to international travel to better understand what causes the increased susceptibility for the acquisition of ESBL producing organisms in the gut. As of now, no studies have analysed the human microbiota of travellers before and after travel, to answer the question if, independent of known risk factors, travel itself causes a dysbiosis in the human gut, and if so, which intermediate factors may be involved in this disturbance (27).

Lastly, the large impact of international travel on ESBL-E endemicity in the Netherlands is relevant for medical specialists, infection prevention specialists, general practitioners and public health practitioners. Implications for each group needs further thought and possibly more research to be able to formulate and manage the implications of international travel on ESBL-E endemicity through specific policies and guidelines.

## REFERENCES


1. UNWTO Tourism Highlights 2018 Edition.
2. CBvdS C. Lange vakanties per land en werelddeel 2002-2013 [Long holidays per country and continent 2002-2013]. Available on request from CBS. .
3. Immigratie- en Naturalisatiedienst. *Ministerie van Justitie en Veiligheid*. Asylum Trends. Monthly Report on Asylum Applications in The Netherlands. March 2019.
4. Woerther PL, Andremont A, Kantele A. Travel-acquired ESBL-producing Enterobacteriaceae: impact of colonization at individual and community level. Journal of travel medicine. 2017;24(suppl_1):S29-s34.
5. ECDC. Surveillance of antimicrobial resistance in Europe 2017. https://ecdc.europa.eu/sites/portal/files/documents/EARS-Net-report-2017-update-jan-2019.pdf.
6. Chmelnitsky I, Carmeli Y, Leavitt A, Schwaber MJ, Navon-Venezia S. CTX-M-2 and a new CTX-M-39 enzyme are the major extended-spectrum beta-lactamases in multiple Escherichia coli clones isolated in Tel Aviv, Israel. Antimicrobial agents and chemotherapy. 2005;49(11):4745-50.
7. CBvdS C. Zomervakanties 2017. https://wwwcbsnl/nl-nl/maatwerk/2018/30/zomervakanties-2017. 2018.
8. Ruppe E, Armand-Lefevre L, Estellat C, Consigny PH, El Mniai A, Boussadia Y, et al. High Rate of Acquisition but Short Duration of Carriage of Multidrug-Resistant Enterobacteriaceae After Travel to the Tropics. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;61(4):593-600.
9. Titelman E, Hasan CM, Iversen A, Naucler P, Kais M, Kalin M, et al. Faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae is common 12 months after infection and is related to strain factors. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014;20(8):O508-15.
10. Barreto Miranda I, Ignatius R, Pfuller R, Friedrich-Janicke B, Steiner F, Paland M, et al. High carriage rate of ESBL-producing Enterobacteriaceae at presentation and follow-up among travellers with gastrointestinal complaints returning from India and Southeast Asia. Journal of travel medicine. 2016;23(2):tav024.
11. van Duijkeren E, Wielders CCH, Dierikx CM, van Hoek A, Hengeveld P, Veenman C, et al. Long-term Carriage of Extended-Spectrum beta-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae in the General Population in The Netherlands. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;66(9):1368-76.
12. Heffernan JM, Smith RJ, Wahl LM. Perspectives on the basic reproductive ratio. Journal of the Royal Society, Interface. 2005;2(4):281-93.
13. Mollema FP, Richardus JH, Behrendt M, Vaessen N, Lodder W, Hendriks W, et al. Transmission of methicillin-resistant Staphylococcus aureus to household contacts. Journal of clinical microbiology. 2010;48(1):202-7.
14. Haverkate MR, Platteel TN, Fluit AC, Cohen Stuart JW, Leverstein-van Hall MA, Thijsen SFT, et al. Quantifying within-household transmission of extended-spectrum beta-lactamase-producing bacteria. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2017;23(1):46.e1-.e7.
15. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M beta-lactamases: temporal and geographical shifts in genotype. The Journal of antimicrobial chemotherapy. 2017;72(8):2145-55.
16. Lundborg CS, Tamhankar AJ. Antibiotic residues in the environment of South East Asia. BMJ (Clinical research ed). 2017;358:j2440.

17. Canton R, Ruiz-Garbajosa P. Co-resistance: an opportunity for the bacteria and resistance genes. Current opinion in pharmacology. 2011;11(5):477-85.
18. Kantele A, Mero S, Kirveskari J, Laaveri T. Fluoroquinolone antibiotic users select fluoroquinolone-resistant ESBL-producing Enterobacteriaceae (ESBL-PE) - Data of a prospective traveller study. Travel medicine and infectious disease. 2017;16:23-30.
19. Ozawa S, Evans DR, Bessias S, Haynie DG, Yemeke TT, Laing SK, et al. Prevalence and Estimated Economic Burden of Substandard and Falsified Medicines in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. JAMA network open. 2018;1(4):e181662.
20. Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. The Lancet Infectious diseases. 2001;1(2):101-14.
21. Youmans BP, Ajami NJ, Jiang ZD, Campbell F, Wadsworth WD, Petrosino JF, et al. Characterization of the human gut microbiome during travelers' diarrhea. Gut microbes. 2015;6(2):110-9.
22. Sheehan D, Moran C, Shanahan F. The microbiota in inflammatory bowel disease. Journal of gastroenterology. 2015;50(5):495-507.
23. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS biology. 2008;6(11):e280.
24. Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, et al. Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome. 2016;4(1):39.
25. Kantele A, Laaveri T, Mero S, Vilkman K, Pakkanen SH, Ollgren J, et al. Antimicrobials increase travelers' risk of colonization by extended-spectrum betalactamase-producing Enterobacteriaceae. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(6):837-46.
26. Reuland EA, Sonder GJ, Stolte I, Al Naiemi N, Koek A, Linde GB, et al. Travel to Asia and traveller's diarrhoea with antibiotic treatment are independent risk factors for acquiring ciprofloxacin-resistant and extended spectrum beta-lactamase-producing Enterobacteriaceae-a prospective cohort study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2016;22(8):731.e1-7.
27. Rasko DA. Changes in microbiome during and after travellers' diarrhea: what we know and what we do not. Journal of travel medicine. 2017;24(suppl_1):S52-s6.
28. Huijbers PM, de Kraker M, Graat EA, van Hoek AH, van Santen MG, de Jong MC, et al. Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in humans living in municipalities with high and low broiler density. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2013;19(6):E256-9.
29. Reuland EA, Al Naiemi N, Kaiser AM, Heck M, Kluytmans JA, Savelkoul PH, et al. Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam. The Journal of antimicrobial chemotherapy. 2016;71(4):1076-82.
30. Wielders CCH, van Hoek A, Hengeveld PD, Veenman C, Dierikx CM, Zomer TP, et al. Extended-spectrum beta-lactamase- and pAmpC-producing Enterobacteriaceae among the general population in a livestock-dense area. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2017;23(2):120.e1-.e8.
31. de Greeff SCaM, J.W. NethMap 2018 Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2017.
32. Meyer E, Gastmeier P, Kola A, Schwab F. Pet animals and foreign travel are risk factors for colonisation with extended-spectrum beta-lactamase-producing Escherichia coli. Infection. 2012;40(6):685-7.

33. Ny S, Lofmark S, Borjesson S, Englund S, Ringman M, Bergstrom J, et al. Community carriage of ESBL-producing Escherichia coli is associated with strains of low pathogenicity: a Swedish nationwide study. The Journal of antimicrobial chemotherapy. 2017;72(2):582-8.
34. van Hoek AH, Schouls L, van Santen MG, Florijn A, de Greeff SC, van Duijkeren E. Molecular characteristics of extended-spectrum cephalosporin-resistant Enterobacteriaceae from humans in the community. PloS one. 2015;10(6):e0129085.
35. Bassyouni RH, Gaber SN, Wegdan AA. Fecal carriage of extended-spectrum beta-lactamase- and AmpC- producing Escherichia coli among healthcare workers. Journal of infection in developing countries. 2015;9(3):304-8.
36. Adler A, Baraniak A, Izdebski R, Fiett J, Salvia A, Samso JV, et al. A multinational study of colonization with extended spectrum beta-lactamase-producing Enterobacteriaceae in healthcare personnel and family members of carrier patients hospitalized in rehabilitation centres. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014;20(8):O516-23.
37. March A, Aschbacher R, Sleghel F, Soelva G, Kaczor M, Migliavacca R, et al. Colonization of residents and staff of an Italian long-term care facility and an adjacent acute care hospital geriatric unit by multidrug-resistant bacteria. The new microbiologica. 2017;40(4):258-63.
38. Leistner R, Meyer E, Gastmeier P, Pfeifer Y, Eller C, Dem P, et al. Risk factors associated with the community-acquired colonization of extended-spectrum beta-lactamase (ESBL) positive Escherichia Coli. an exploratory case-control study. PloS one. 2013;8(9):e74323.
39. de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, et al. Dissemination of cephalosporin resistance genes between Escherichia coli strains from farm animals and humans by specific plasmid lineages. PLoS genetics. 2014;10(12):e1004776.
40. Dorado-Garcia A, Smid JH, van Pelt W, Bonten MJM, Fluit AC, van den Bunt G, et al. Molecular relatedness of ESBL/AmpC-producing Escherichia coli from humans, animals, food and the environment: a pooled analysis. The Journal of antimicrobial chemotherapy. 2018;73(2):339-47.
41. Huizinga P, van den Bergh MK, van Rijen M, Willemsen I, van 't Veer N, Kluytmans J. Proton Pump Inhibitor Use Is Associated With Extended-Spectrum beta-Lactamase-Producing Enterobacteriaceae Rectal Carriage at Hospital Admission: A Cross-Sectional Study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(3):361-3.
42. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. Gut. 2016;65(5):740-8.
43. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, Poole AC, et al. Proton pump inhibitors alter the composition of the gut microbiota. Gut. 2016;65(5):749-56.
44. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature. 2018;555(7698):623-8.
45. van der Steen M, Leenstra T, Kluytmans JA, van der Bij AK. Trends in Expanded-Spectrum Cephalosporin-Resistant Escherichia coli and Klebsiella pneumoniae among Dutch Clinical Isolates, from 2008 to 2012. PloS one. 2015;10(9):e0138088.
46. Huizinga P, Schrauwen E, Garcia-Cobos S, Willemsen I, Verhulst C, Friedrich AW, et al. Extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) isolated from bean sprouts in the Netherlands. PloS one. 2018;13(8):e0203338.
47. van der Bij AK, Pitout JD. The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. The Journal of antimicrobial chemotherapy. 2012;67(9):2090-100.
48. World Health Organization. GLOBAL ACTION PLAN ON ANTIMICROBIAL RESISTANCE. 2015.

49. World Health Organization. Central Asian and Eastern European Surveillance of Antimicrobial Resistance. Annual report 2014.
50. Girlich D, Bouihat N, Poirel L, Benouda A, Nordmann P. High rate of faecal carriage of extended-spectrum beta-lactamase and OXA-48 carbapenemase-producing Enterobacteriaceae at a university hospital in Morocco. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014;20(4):350-4.
51. Epelboin L, Robert J, Tsyrina-Kouyoumdjian E, Laouira S, Meyssonnier V, Caumes E. High Rate of Multidrug-Resistant Gram-Negative Bacilli Carriage and Infection in Hospitalized Returning Travelers: A Cross-Sectional Cohort Study. Journal of travel medicine. 2015;22(5):292-9.
52. Osthoff M, McGuinness SL, Wagen AZ, Eisen DP. Urinary tract infections due to extended-spectrum beta-lactamase-producing Gram-negative bacteria: identification of risk factors and outcome predictors in an Australian tertiary referral hospital. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2015;34:79-83.
53. Soraas A, Sundsfjord A, Sandven I, Brunborg C, Jenum PA. Risk factors for community-acquired urinary tract infections caused by ESBL-producing enterobacteriaceae--a case-control study in a low prevalence country. PloS one. 2013;8(7):e69581.
54. Laupland KB, Church DL, Vidakovich J, Mucenski M, Pitout JD. Community-onset extended-spectrum beta-lactamase (ESBL) producing Escherichia coli: importance of international travel. The Journal of infection. 2008;57(6):441-8.
55. Patel U, Dasgupta P, Amoroso P, Challacombe B, Pilcher J, Kirby R. Infection after transrectal ultrasonography-guided prostate biopsy: increased relative risks after recent international travel or antibiotic use. BJU international. 2012;109(12):1781-5.
56. Williamson DA, Masters J, Freeman J, Roberts S. Travel-associated extended-spectrum beta-lactamase-producing Escherichia coli bloodstream infection following transrectal ultrasound-guided prostate biopsy. BJU international. 2012;109(7):E21-2.
57. Dubinsky-Pertzov B, Temkin E, Harbarth S, Fankhauser-Rodriguez C, Carevic B, Radovanovic I, et al. Carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae and the risk of surgical site infection after colorectal surgery: a prospective cohort study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018.
58. Bert F, Larroque B, Paugam-Burtz C, Dondero F, Durand F, Marcon E, et al. Pretransplant fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae and infection after liver transplant, France. Emerging infectious diseases. 2012;18(6):908-16.
59. Antibioticabeleid SW. SWAB Richtlijn Peri-operatieve profylaxe juni 2018.
60. Froding I, Valik JK, Bolinder L, Naucler P, Giske CG. Prediction of bloodstream infection caused by ESBL-producing Enterobacterales in patients with suspected community-onset sepsis. International journal of antimicrobial agents. 2019.
61. Rottier WC, Bamberg YR, Dorigo-Zetsma JW, van der Linden PD, Ammerlaan HS, Bonten MJ. Predictive value of prior colonization and antibiotic use for third-generation cephalosporin-resistant enterobacteriaceae bacteremia in patients with sepsis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;60(11):1622-30.
62. Dall LB, Lausch KR, Gedebjerg A, Fuursted K, Storgaard M, Larsen CS. Do probiotics prevent colonization with multi-resistant Enterobacteriaceae during travel? A randomized controlled trial. Travel medicine and infectious disease. 2019;27:81-6.
63. Maurine a. Leverstein-van Hall JCS, Guido M. Voets, Dik Versteeg, Evelien Roelofsen en ad C. Fluit Carbapenem-resistente Klebsiella pneumoniae na verblijf in het buitenland. NED TIJDSCHR GENEESKD 2010;154:A20132.

64. Potron A, Kalpoe J, Poirel L, Nordmann P. European dissemination of a single OXA-48-producing Klebsiella pneumoniae clone. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2011;17(12):E24-6.
65. Ruppe E, Armand-Lefevre L, Estellat C, El-Mniai A, Boussadia Y, Consigny PH, et al. Acquisition of carbapenemase-producing Enterobacteriaceae by healthy travellers to India, France, February 2012 to March 2013. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2014;19(14).
66. Kuenzli E, Jaeger VK, Frei R, Neumayr A, DeCrom S, Haller S, et al. High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli in Swiss travellers to South Asia- a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. BMC infectious diseases. 2014;14:528.
67. Kelly AM, Mathema B, Larson EL. Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. International journal of antimicrobial agents. 2017;50(2):127-34.
68. Nicolas-Chanoine MH, Vigan M, Laouenan C, Robert J. Risk factors for carbapenem-resistant Enterobacteriaceae infections: a French case-control-control study. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2019;38(2):383-93.
69. Manenzhe RI, Zar HJ, Nicol MP, Kaba M. The spread of carbapenemase-producing bacteria in Africa: a systematic review. The Journal of antimicrobial chemotherapy. 2015;70(1):23-40.
70. Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. Epidemiology of carbapenemase-producing Enterobacteriaceae and Acinetobacter baumannii in Mediterranean countries. BioMed research international. 2014;2014:305784.
71. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014;20(9):821-30.
72. Myat TO, Hannaway RF, Zin KN, Htike WW, Win KK, Crump JA, et al. ESBL- and Carbapenemase-Producing Enterobacteriaceae in Patients with Bacteremia, Yangon, Myanmar, 2014. Emerging infectious diseases. 2017;23(5):857-9.
73. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet Infectious diseases. 2010;10(9):597-602.
74. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. The Lancet Infectious diseases. 2011;11(5):355-62.
75. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet Infectious diseases. 2016;16(2):161-8.
76. Tian GB, Doi Y, Shen J, Walsh TR, Wang Y, Zhang R, et al. MCR-1-producing Klebsiella pneumoniae outbreak in China. The Lancet Infectious diseases. 2017;17(6):577.
77. Terveer EM, Nijhuis RHT, Crobach MJT, Knetsch CW, Veldkamp KE, Gooskens J, et al. Prevalence of colistin resistance gene (mcr-1) containing Enterobacteriaceae in feces of patients attending a tertiary care hospital and detection of a mcr-1 containing, colistin susceptible E. coli. PloS one. 2017;12(6):e0178598.
78. Nijhuis RH, Veldman KT, Schelfaut J, Van Essen-Zandbergen A, Wessels E, Claas EC, et al. Detection of the plasmid-mediated colistin-resistance gene mcr-1 in clinical isolates and stool specimens

obtained from hospitalized patients using a newly developed real-time PCR assay. The Journal of antimicrobial chemotherapy. 2016;71(8):2344-6.

79. Kluytmans-van den Bergh MF, Huizinga P, Bonten MJ, Bos M, De Bruyne K, Friedrich AW, et al. Presence of mcr-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2016;21(9):30149.
80. El Garch F, de Jong A, Bertrand X, Hocquet D, Sauget M. mcr-1-like detection in commensal Escherichia coli and Salmonella spp. from food-producing animals at slaughter in Europe. Veterinary microbiology. 2018;213:42-6.
81. Schrauwen EJA, Huizinga P, van Spreuwel N, Verhulst C, Kluytmans-van den Bergh MFQ, Kluytmans J. High prevalence of the mcr-1 gene in retail chicken meat in the Netherlands in 2015. Antimicrobial resistance and infection control. 2017;6:83.
82. Grami R, Mansour W, Mehri W, Bouallegue O, Boujaafar N, Madec JY, et al. Impact of food animal trade on the spread of mcr-1-mediated colistin resistance, Tunisia, July 2015. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2016;21(8):30144.
83. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. The Lancet Infectious diseases. 2013;13(12):1057-98.
84. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC pediatrics. 2010;10:39.
85. Parathon H, Kuntaman K, Widiastoety TH, Muliawan BT, Karuniawati A, Qibtiyah M, et al. Progress towards antimicrobial resistance containment and control in Indonesia. BMJ (Clinical research ed). 2017;358:j3808.
86. Dondorp AM, Limmathurotsakul D, Ashley EA. What's wrong in the control of antimicrobial resistance in critically ill patients from low- and middle-income countries? Intensive care medicine. 2018;44(1):79-82.
87. Livermore DM. The 2018 Garrod Lecture: Preparing for the Black Swans of resistance. The Journal of antimicrobial chemotherapy. 2018;73(11):2907-15.
88. Paltansing S, Vlot JA, Kraakman ME, Mesman R, Bruijning ML, Bernards AT, et al. Extended-spectrum beta-lactamase-producing enterobacteriaceae among travelers from the Netherlands. Emerging infectious diseases. 2013;19(8):1206-13.