Summarizing discussion and future directions
SUMMARIZING DISCUSSION

Resistance to beta-lactam antibiotics due to carriage of ESBL-producing Enterobacteriaceae (ESBL-E) is increasing at an alarming rate, worldwide, not only in hospitalized patients but also in the community. Percentages of carriage of ESBL range from about 7% in Europe up to nearly 70% in some other parts of the world.1 Not only ESBLs are found but also carbapenemases are being detected with increasing frequency.

With respect to antibiotic resistance in general, and to ESBL-E in particular, the Netherlands appears as an island of low prevalence compared to other European regions. It is a country with low and prudent use of antimicrobials in the human population. However, due to several risk factors the prevalence may well be on the verge of a substantial increase. International travel for example may contribute to the acquisition and therefore introduction of resistant strains. Also use of antimicrobials -one of the main problems, due to its huge impact on emerging resistance- increases in the Netherlands due to the rise in immunocompromised patients, in use of invasive therapies and foreign bodies. Obtaining an estimate of the prevalence of ESBL-producing strains is important as a starting point for infection control policies and to establish empirical regimens for antimicrobial therapy. An increase in prevalence may necessitate adjustments in antimicrobial strategies.

For a proper assessment of the situation, it is, first of all, important to determine the prevalence of ESBL-E in the community in the Netherlands. Before 2000, ESBL-E was recorded in <1% among hospitalized patients in Dutch hospitals. After 2005 two studies reported an increase of this prevalence to 6 - 8%.2,3 These studies were performed more than ten years ago in hospitalized patients. Later on, in 2011, a high percentage of carriage of ESBL-producing bacteria on admission, nearly 5%, pointed towards the possible existence of a community reservoir.4

Before the start of a large study in the Dutch open community to determine the prevalence of carriage of ESBL-E, a pilot study was conducted to obtain an estimate of this percentage (Chapter 2). Therefore, we focused on Dutch primary care patients with presumed gastrointestinal discomfort. Unexpectedly, a high percentage (10.1%) of carriage was found. The participants included in this study were from two regions in the Netherlands: a primary care population in the region of Amsterdam (a densely populated urban area) and Brabant (a more rural area). Although two completely different regions in the Netherlands were taken, the study still involved a select population because these patients all had gastrointestinal complaints. According to the guidelines for the Dutch general practice, diagnostics by fecal culture is only requested for patients with gastrointestinal complaints that last for more than 10 days or gastrointestinal complaints after visiting foreign countries, especially the (sub)tropics.5 One of the major aims of the research carried out was to identify risk factors for the acquisition of resistant strains. In this pilot study no risk factors could be analyzed.
because no such data were available. There was for example no information on travel for these patients. However, it seems likely that visiting foreign countries is responsible for at least part of the prevalence of ESBL-E in Dutch outpatients, in view of the algorithms laid down in the professional standards for general practitioners mentioned above. Of the resistant strains found the ESBL-encoding genes were characterized, and strain typing was performed to gain insight in the epidemiology and to identify any genetic relatedness. We detected predominantly ESBL-producing *E. coli*, with CTX-M-15 as the most frequent ESBL type. This is comparable to the epidemiology in the community worldwide. Assessing our results from the latter perspective, note that in several countries the expansion of CTX-M-15-producing *E. coli* is due to the worldwide pandemic clone ST131, a more virulent clone that is associated with more severe infections. In contrast, the *E. coli* strains that we identified belonged to multiple sequence type clonal complexes and the presence of CTX-M-15 was scattered over different clusters; so, we could not confirm these data with our research. An important feature of ESBL-producing Enterobacteriaceae is co-resistance to other antibiotics. Co-resistance is associated with carriage of ESBL-E due to genes encoding for other plasmid-mediated resistance to other classes of antibiotics. In this study the problem of multiresistance was a significant problem as well: 45% was resistant to at least one agent in three or more antimicrobial categories and therefore likely to cause therapeutic failure. Twelve percent of the ESBL-E was resistant to gentamicin, ciprofloxacin and cotrimoxazole. Because this first study showed emerging resistance by ESBL-E in just a subset of the Dutch outpatient population, it was interesting to find out the prevalence in the overall Dutch community.

To this end, a cross-sectional study was performed to determine prevalence and risk factors (Chapter 3). We initially assumed that the prevalence of carriage in the general adult population would be lower than in a specific subset of patients with gastrointestinal symptoms. With a percentage of 8.6% of ESBL-E carriage, the data from the general population confirmed the pilot study. In conclusion, the rate of ESBL carriage was still higher than expected and therefore identifying risk factors even more valuable. Several studies report risk factors in hospitalized patients such as diabetes, antimicrobial use and comorbidity. However, these risk factors cannot be applied to the more healthy community. Unfortunately, fewer studies have been conducted where risk factors were identified in the community; only a few European studies are available. In general, one of the main problems in studies focusing on antibiotic resistance in the community, is how to obtain a true representation of the community together with a reasonable sample size, as both are necessary to obtain a reliable answer. In this study we did obtain true community-derived isolates because participants were randomly selected from databases of general practices affiliated at the Academic General Practice Network (AGPN), VU University Medical Center, Amsterdam. In the Netherlands, health insurance is obligatory and all inhabitants have to
be registered with a general practitioner, regardless of their health status. The database therefore is a representative sample of the general population.

We found that in this community setting the main risk factors were antibiotic use, use of gastric acid-suppressing medication, and travel to Africa, Asia or the USA. Additional risk factors were having a mother born in Asia and possibly working as a cabin crew member for an airline. The effects of antibiotic use and travel to Asia and Africa are not unexpected, and also frequently reported in other studies. Two other risk factors we found, however, stood out: the effects of antacid use and the more than threefold risk associated with travel to the USA have not been clearly shown before. The role of acid suppression has been noticed before, but received little attention. An association between antacid use and colonization with ESBL-E seems plausible and might be due to the mechanism of a disrupted barrier due to an increased gastric pH, and therefore diminished defense system. The risk of antacid use is particularly important, because antacids are used widely, and because it also points to ingestion as a route of acquisition of ESBL-E. This is in line with other reports suggesting the food chain as a possible source of resistance genes.

Our findings, combined with previous studies that show an abundant presence of ESBL-E in the food chain, warrant more attention to the potential risk to public health of resistant microorganisms in food and water. Referring to travel again, an advantage of our approach (i.e. using the general practitioner’s databases to draw a sample from the general population) is that we did not select for persons attending a travel clinic, which introduces strong bias towards countries that require vaccination or malaria prophylaxis. Our study comprised a cross-sectional study design with all WHO regions included in the analysis, and so we could identify Northern America or Northern Africa as high-risk areas. In addition to ESBL also one case of carbapenemase, OXA-48, was detected in a participant that had recently visited the USA and Egypt. Egypt is well-known for the presence of OXA-48.

Since travel appeared as a major risk factor for ESBL-E acquisition, we also performed a study in travelers (Chapter 4). In this study we investigated the rate of and risk factors for travel-related acquisition of ESBL-producing Enterobacteriaceae (ESBL-E), ciprofloxacin-resistant Enterobacteriaceae (CIPR-E) and carbapenem-resistant Enterobacteriaceae (CR-E). For ESBL-E this rate increased from 6.1% pre-travel to 23.4% post-travel, comparable with data published in other studies. For CIPR-E the rate increased from 10.1% to 32.5% respectively. Import of quinolone resistance genes by travel was seen in one third to half of travelers in another study. In our prospective cohort study one carbapenemase-producing isolate, OXA-48, was acquired, also after a visit to Egypt and in line with more studies showing the acquisition of CR-E in asymptomatic travelers. The presence of traveler’s diarrhea and use of antimicrobials are found as risk factors for acquisition of resistance in other studies. The present study shows an increased risk of acquisition of ESBL-E as well as CIPR-E in travelers with diarrhea and a highly increased risk in those travelers that developed traveler’s diarrhea.
and used antimicrobial agents. In conclusion, international travel to (sub) tropical areas, especially travel to Asia, and diarrhea combined with antimicrobial use are important risk factors for acquiring ESBL-E and CIPR-E. Findings from ours and other studies we mentioned suggest that routinely prescribing (stand-by) antibiotics for traveler’s diarrhea should be strongly reconsidered, also because traveler’s diarrhea is usually self-limiting.23

Our study in the community pointed to Northern Africa as a region that brings a risk for ESBL-E acquisition. We had the opportunity to study the prevalence of ESBL-E, both in Egyptian patients and in Egyptian meat. These studies showed that the problem in Egypt is quite substantial. ESBL production was detected in nearly half of the Enterobacteriaceae causing bloodstream infections (Chapter 5). With respect to food in Egypt, nearly two thirds of chicken meat samples were contaminated with ESBL-E, and, even worse, around 10% of meat samples were also contaminated with carbapenemase producers (Chapter 6). Also a very interesting issue is the possible transmission and acquisition of resistant strains via the food chain. In our study in the community, we found that CTX-M-1-encoding genes, associated with poultry, were not associated with travel while CTX-M-14 and CTX-M-15 were. This suggests that the CTX-M-1-encoding genes were acquired in the Netherlands, with food as a possible source. Several Dutch studies pose an association between ESBL-encoding genes in poultry and those found in humans.24–26 We detected ESBL genes also in Enterobacteriaceae recovered from vegetables (Chapter 7). Six percent of the samples we tested were contaminated by ESBL-E, and the genes found were comparable to what is found in enterobacterial strains from human origin. This finding, also points to the food chain as a possible source of resistance genes. However, a study design to prove the association of acquisition of resistant strains and the food chain is far from easy. Because ESBL-E are found in poultry and meat, one could envisage a study in vegetarians, but, since we detected ESBL-E also in vegetables such a study would not provide a good estimate of the risk associated with eating meat.

An important question is whether the very large amount of antibiotics used in livestock in the Netherlands does play a role in the high prevalence of ESBL-E carriage found in the Dutch community. In the past years, the amount of antibiotics used in poultry decreased substantially, due to a new policy introduced by the Dutch government in 2010. At that time, the Ministry of Agriculture, Nature and Food Quality decreed that antibiotic prescriptions had to be reduced by 50% within five years. In 2011 a further reduction to only 20% was imposed by what is now the Ministry of Economic Affairs, Agriculture and Innovation. By 2015 total sales of antimicrobial veterinary medicinal products have dropped to 207.000 kg/year, indeed a reduction of approximately 50% compared to total sales in 2010 (MARAN report 2015).27 A study by Kluymans et al. suggests that the percentage of CTX-M-1 decreased the last few years, possibly as a consequence of reduced antibiotic use in food animals.28
Influx from hospitals to the community is also an important source of resistant strains.\textsuperscript{12,29} Transmission in the community can occur by e.g. persistent carriers of resistant strains to household members. Several studies describe person-to-person transmission of resistant strains. In the study on ESBL-E carriage in the community, we too described three households where two members were carriers. The presence of different strains and plasmids in two households suggests that acquisition of ESBL-E within households is not only due to strain transmission.

Travel appeared as a source of carbapenemase-producing strains, however only very few were detected in the community or after travel. Influx, however, can occur with patients that have been treated in foreign hospitals (Chapter 8). We described an NDM-1-producing \textit{Klebsiella pneumoniae} that was imported from the Balkan region, and found in a patient hospitalized in the east of the Netherlands. The same strain was detected in another patient during her stay in the same hospital as the index case. The strains were shown to be identical by amplified-fragment length polymorphism (AFLP). Halting the spread of resistance starts with its detection, but an important question is what type of detection method is most cost-effective. Our study favors the use of molecular methods (Chapter 9).

One resistance mechanism has been quite neglected so far. Plasmidal AmpC (pAmpC) has not been registered yet as a public health problem with consequences. Since pAmpC-producing isolates were not identified as ESBL producers by routine algorithms, a consistent risk is present that due to their plasmidal location, further increase of their prevalence might go undetected. The crucial point for determining prevalence and risk factors, and therefore impact on public health, is accurate detection in the laboratory. The Dutch national guidelines report screening and confirmation of ESBLs and carbapenemases. However, the detection of pAmpC remains difficult. In our study several phenotypic tests were evaluated and we found that the best method is to screen for reduced susceptibility to third generation cephalosporins combined with reduced susceptibility to cefoxitin. Subsequent confirmation via a combination disk diffusion test using cloxacillin was shown to have the best sensitivity and specificity in relation to costs (Chapter 10). We then measured the prevalence of carriage of Enterobacteriaceae producing pAmpC in the community, and this proved to be low, at a rate of 1.3%. This is difficult to compare with other studies because, to the best of our knowledge, no studies on pAmpC in the community have been performed so far. Furthermore, risk factors were difficult to analyze due to the small study population. However, within this small sample size we found admission to a hospital in the previous year as the only risk factor (Chapter 11).
CONCLUSION AND FUTURE DIRECTIONS

In summary, resistant genes are present in humans, animals, food and the environment. Resistance due to beta-lactamases has been linked to several known and unknown pathways and reservoirs. What was first mainly a hospital problem is now emerging in the community and several risk factors have been identified. The food chain may play an increasing role, in addition to e.g. the increase in international travel and medical tourism. Hence, also in the Netherlands, a country with a low consumption rate of antibiotics in humans, resistant strains are more and more frequently detected. Not only ESBLs are a concern, but also carbapenemases and plasmidal AmpC. Reliable detection methods are crucial to detect carriers of resistant strains, and to be able to take adequate measures for infection control in order to restrain further spread. Identification of risk factors can contribute to improving empiric therapy in an essential way, minimizing therapeutic failure and thereby reducing morbidity and mortality.

On the basis of the results presented in this thesis we feel that important questions to ask when a patient is admitted to a hospital with severe infection and suspicion of Gram-negative bacterial cause are:

Over the last year:
- Did you travel?
- Did you use antibiotics?
- Did you use antacids?
REFERENCES


