General introduction and outline of this thesis

INTRODUCTION

A major breakthrough in medicine was the discovery of penicillin in 1929 by Alexander Fleming (1). In 1940 the world celebrated as its clinical efficacy was demonstrated for the first time. In the same year however, a study was published which showed bacteria could produce an enzyme that inactivated penicillin (2), but this early warning was largely ignored.

Following a renewed warning by Calvin Kunin in 1993 (3) antimicrobial resistance has increasingly become recognized as a major global health problem over the past 25 years. Overuse of antimicrobials and lack of sanitation and infection control has led to rapidly increasing rates of antimicrobial resistance due to selection of resistant clones and their spread locally, regionally and often times worldwide. Recent world-wide estimates are that there are already 700.000 deaths annually due to antimicrobial resistance, and it is predicted that, without effective interventions, this number will further increase to 10 million deaths annually by the year 2050. The strongest impact of antimicrobial resistance emergence is predicted to occur in Asia and Africa where annual death rates are estimated to approach 5 million and 4 million by the year 2050, respectively. However, also Europe is already affected and may experience an estimated number of 400.000 deaths yearly by 2050 due to diseases caused by antimicrobial resistant micro-organisms (4). Although these estimates have been criticized for overestimating mortality resulting from antimicrobial resistance (5), there is no doubt that there is a large and increasing burden of antimicrobial resistance on clinical and public health that needs to be addressed.

Antibiotic-resistant Enterobacteriaceae

Among bacteria belonging to the family of Enterobacteriaceae resistance to beta-lactam antibiotics has been emerging worldwide. The family of Enterobacteriaceae consists of multiple species of Gram-negative bacilli, several of which are part of the normal human microbiota, especially in the gut. These same species are also important causes of community-acquired and nosocomial infections. Enterobacteriaceae can acquire resistance genes through horizontal transfer of mobile genetic elements like plasmids. Among the genes thus transferred may be those that encode for the production of enzymes called extended-spectrum beta-lactamases (ESBLs). ESBLs have broad-spectrum activity against penicillins, cephalosporins and monobactams, which they degrade by hydrolyzing the beta-lactam ring of these antibiotics, leading to inactivation. During the 1990s the first recognized ESBLs were the so called TEM and SHV genes, which were mostly carried by *K. pneumoniae* strains causing hospital infection. Over time, the epidemiology of ESBLs shifted towards CTX-M genes which were carried by *E. coli* originating from the community. Due to their rapid spread and increase around

the world the phenomenon has been referred as the "CTX-M pandemic" (6). CTX-M-15 (part of CTX-M group 1) and CTX-M-14 (part of CTX-M group 9) are the most prevalent ESBL genotypes (7). CTX-M-15 has spread worldwide and is the dominant ESBL-gene in most regions. CTX-M-14 is dominant in China, South-Korea, Japan and Spain (8). The only class of beta-lactam antibiotics that are relatively resistant to degradation by ESBLs are the carbapenems, including imipenem and meropenem; they are often used to treat infections due to ESBL-producing Gram-negative bacteria. However, Enterobacteriaceae can acquire resistance genes encoding for enzymes called carbapenemases. These carbapenemase-producing Enterobacteriaceae (CPE's) are resistant to most betalactam antibiotics, including the carbapenems. There are different groups of enzymes with the capability to inactivate carbapenems. The carbapenemases which have now spread worldwide are KPC (*Klebsiella pneumoniae* carbapenemases), NDM (New Delhi metallo-beta-lactamase), OXA-48 (oxacillinases), IMP (imipenemase) metallo-β-lactamase and VIM (Verona integron–encoded metallo-β-lactamase). Geographically, the primary reservoirs or sites of emergence of the carbapenemases have been the USA, Israel, Greece and Italy for KPC, the Indian subcontinent for NDM, Turkey and North Africa for OXA-48 and Greece, Taiwan and Japan for VIM, and the Asia Pacific for IMP (9, 10).

In addition, multiple genes encoding for resistance to other antibiotic classes such as quinolones and aminoglycosides are often located on the same plasmid (11, 12). Therefore, ESBL-E and CPE-E are often resistant to multiple antibiotic classes. Multidrug resistant Enterobacteriaceae leave doctors with few to no effective antimicrobial agents for the prevention and treatment of infections with these bacteria. Consequently, older drugs including polymyxin class antibiotics (polymyxin B and polymyxin E [colistin]), which were largely disregarded in the past due to their (nephro)toxic side effects, have made a comeback and are now prescribed as a last resort treatments for severe infections with multidrug resistant Enterobacteriaceae. Although it was long thought that only chromosomal mutations could code for colistin resistance, the 2015 discovery in China of a plasmid based colistine resistance gene, designated mrc-1, raised serious concerns (13). Soon after its discovery, many reports described the presence of the mcr-1 gene in isolates from animals, animal food products, humans and environmental samples from around the world. Reports on carbapenemase-producing Enterobacteriaceae that have acquired the mcr-1 gene worries the scientific and medical community as it could lead to the emergence of untreatable so called pandrug resistant Enterobacteriaceae (14). Unfortunately, innovative antimicrobial treatment options are few and the current rate of development of new antibiotics seems insufficient to keep up with the emergence and spread of antimicrobial resistance (15).

Antibiotic-resistant Enterobacteriaceae and travel

The overuse of antibiotics in animals and humans can lead to high endemic levels of ESBL-E locally, through selective pressure (16-18). Community carriage rates are high in regions like South-East Asia, Western Pacific and Africa (Figure 1). In addition to overuse, limited access to proper sanitation facilities and contamination of surface waters used for irrigation of crops, and of drinking water supplies facilitate the spread of ESBL-E and contribute to the level of endemicity of ESBL-E in these countries (Figure 2) (8, 19). Moreover, the overuse of antibiotics of multiple classes leads to accumulation of multiple resistance genes in the environment, which can subsequently be acquired by people (20). In such a situation, the use of just a single antibiotic may be sufficient to select for multidrug resistant isolates, a process called co-selection which drives the emergence of multidrug resistant isolates (21, 22).

Another potential mode of international spread of antimicrobial resistance is through the transport of contaminated foods, livestock and, last but not least, by national and international travel of people. Travellers visiting countries with high, endemic, levels of antimicrobial resistance can acquire bacteria or plasmids carrying resistance genes through contact with indigenous people, food or the environment and import them to their home country. As the human gut microbiota act as a reservoir for antimicrobial resistance genes, international travellers may substantially contribute to the emergence and spread of ESBL-E in their home countries. Given the enormous growth in the number of international travellers, from 25 million in 1950 to 1.326 billion in 2017 (23), it is important to asses to what extent foreign travel poses a risk for the acquisition and spread of antimicrobial resistance. More insights into the rates and determinants of acquisition, persistence and transmission of travel-associated antibiotic-resistant Enterobacteriaceae are needed. These new insights may lead to adjustments of infection prevention guidelines and empiric antibiotic treatment policies to prevent spread and optimize clinical care for the individual patient.

Antibiotic-resistant Enterobacteriaceae in the Dutch community

Since the 1980s cephalosporins have been widely used to treat a wide range of infections including those caused by members of the Enterobacteriaceae. Through selective pressure the ESBL carrying pathogens, resistant to the third generation cephalosporins often prescribed in hospitals, emerged. At first ESBL-infections were limited to hospital acquired infections, but nowadays ESBL genes have accumulated in community pathogens as well, most notably in the species *E. coli* (24). Community-acquired urinary tract and bloodstream infections caused by ESBL-*E. coli* have emerged in the past decade (25-27). Advancing age, urban living, health care contacts and international travel have been among the first risk factors identified for community acquired ESBL-E infections in a Canadian setting (28).

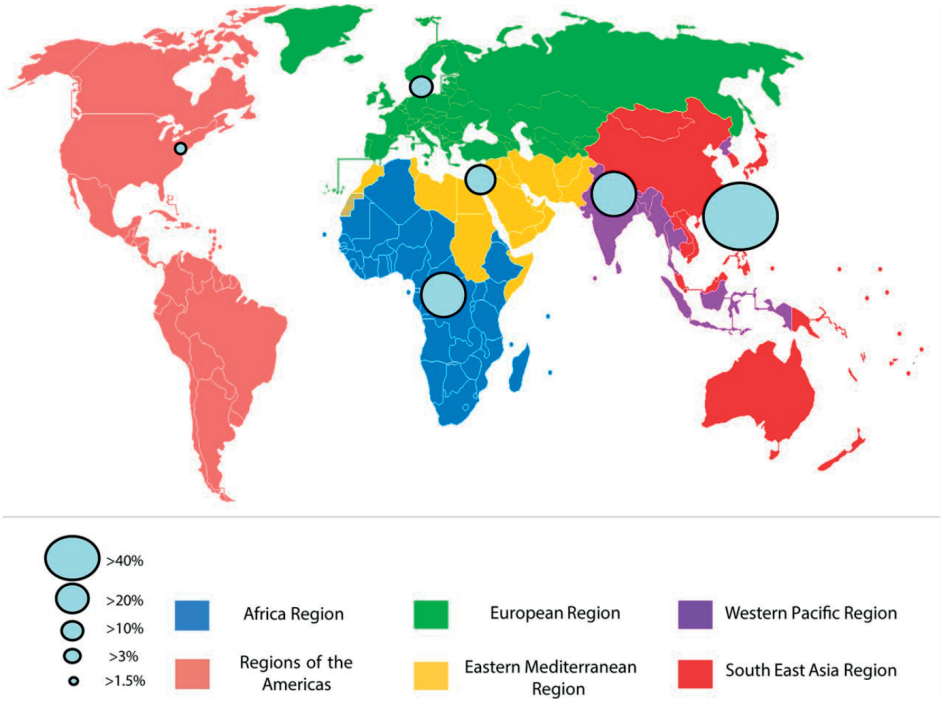

Figure 1. Pooled prevalence of fecal colonization of healthy individuals with extended-spectrum beta-lactamase (ESBL)–producing organisms per World Health Organization region. Circle size represents the ESBL colonization rates (19).

The increasing prevalence of ESBL carriage in the community at large, even in countries with restricted use of antibiotics like the Netherlands, is of great concern, as ESBL-E carriage has been associated with an increased risk of subsequent ESBL-E infections (29, 30). As most studies focus on risk-factors for ESBL-infection in hospitalized patients or outpatients and only few studies have investigated risk factors for ESBLs in healthy adults, predictive factors for ESBL-E carriage in the community are not well defined (19, 31-38). Identifying individuals at risk of ESBL-E acquisition and carriage enables to identify the origin for ESBL-E carriage in the community and enables to foresee public health risks and act accordingly.

AIM OF THE RESEARCH AND OUTLINE OF THIS THESIS

The main aim of the research described in this thesis was to determine the impact of intercontinental travel on the prevalence of antibiotic-resistant Enterobacteriaceae, especially ESBL-producing strains, in the gut of healthy citizens living in the Netherlands.

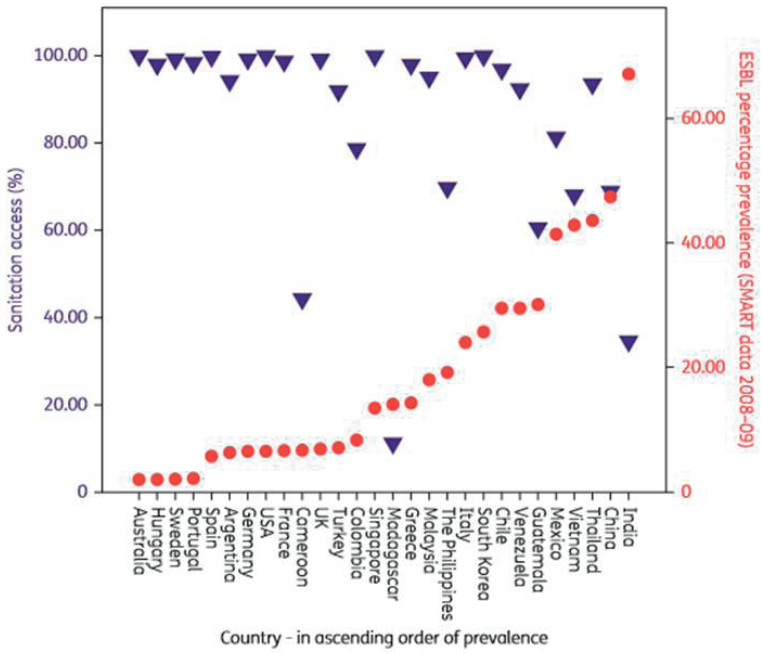

Figure 2. Sanitation access and ESBL prevalence (8).

Moreover, we aimed to determine the subsequent persistence of travel-acquired antibiotic-resistant Enterobacteriaceae after returning home from travel, and the likelihood of their spread from returning travellers to other members of the Dutch population. Potential risk factors for the acquisition during travel and for the persistence after travel of such strains were studied as well.

Travel and acquisition of antibiotic-resistant Enterobacteriaceae

Travel and acquisition of Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E)

In **chapter 2** we summarize findings from previous small to medium sized prospective cohort studies among returning travellers which investigated ESBL acquisition and associated risk factors. These previous studies have reported frequent acquisition of ESBL-E associated with various predictors and sporadic acquisition of CPE among international travellers. However, data on duration of ESBL-E colonisation after travel and assessment of associated predictors for sustained carriage and onward transmission within households were, partly due to the size of many of these studies, very limited.

Therefore, we performed a more definitive large scale study addressing these issues, a study that was named the COMBAT (Carriage of Multiresistant Bacteria After Travel)-

study. In **chapter 3,** we describe the methodology of this large-scale longitudinal cohort study of travellers, the aims of which were:

1. to determine the acquisition rate of ESBL- and carbapenemase-producing Enterobacteriaceae during foreign travel by comparing their prevalence in pre- and post-travel faecal samples
2. to ascertain the duration of carriage of these microorganisms (or their resistance genes/mobile genetic elements) by studying faecal specimens at regular intervals up to 1 year after the travellers had returned to the Netherlands.
3. to mathematically model the decolonization and transmission rates of these imported Enterobacteriaceae (or their resistance genes/mobile elements) within households by prospectively studying consecutive specimens from household members
4. to identify the risk factors for acquisition and persistence of carriage

In **chapter 4** we describe the main results of the COMBAT-study, focusing on ESBL-E acquisition by healthy international travellers and predicitive factors associated with acquisition of ESBL-E, the duration of travel-acquired ESBL-E colonization of travellers once back home and risk factors associated with persistent carriage, and the rate of onward transmission of travel-acquired ESBL-E to household members of these travellers.

Pre-travel carriage of ESBL-E by study participants

In **chapter 5** we investigated the prevalence of ESBL-E and the predictive factors for ESBL-E carriage in our cohort of travellers (and their household members) prior to their travel abroad. To gain more insight in the molecular epidemiology and their resistance phenotype prior to travel, we determined and compared the genotypes and the co-resistance profiles of ESBL-E isolated from pre- and post-travel faecal samples. The hypothesis was that carriage of ESBL-E strains before travel could, to a substantial degree, be attributed to prior international travel of the participants enrolled in the COMBAT study.

Travel and Carbapenemase-producing Enterobacteriaceae (CPE)

In **chapter 6** we focus on the acquisition, persistence and potential transmission of CPE in the same cohort of travellers. Although CPE prevalence in the gut microbiota of healthy community dwellers is much lower, the hypothesis was that, due to the large size of the COMBAT study, at least some participants in the COMBAT study would acquire a CPE during their intercontinental travel, especially if to regions of the world where such strains have emerged in the past, and import CPE strains into the Netherlands.

Travel and acquisition of Plasmid mediated colistin-resistant Enterobacteriaceae

In chapter 7 we describe the acquisition of the mcr-1 gene by a few participants in our study cohort. In order to understand the dynamics behind the worldwide spread of the mcr-1 gene, we subsequently determined the population structure of *E. coli* and the mobile genetic elements carrying the mcr-1 gene by reviewing and comparing whole-genome sequences and MLST profiles from our own travel-acquired mcr-1 carrying isolates and those available from publicly databases and the literature (**chapter 8**).

Travel and acquisition of diarrhoeagenic bacteria, enteral viruses and parasites

Limited prospective data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by healthy individuals during travel. We, therefore, exploited our cohort to study this issue and expand our knowledge in this respect. In **chapter 9** we describe the frequency of travel associated acquisition of eight viral pathogens, six bacterial enteric pathogens and five parasite species in a random selection of travellers by using sensitive PCR-based assays.

In **chapter 10** we present a Summarizing general discussion and present our future perspectives on the topic of emerging antimicrobial resistance in relation to international travel.

REFERENCES

1. Fleming A. On the antibacterial action of cultures of a Penicillium, with special reference to their use in isolation of B.influenzae. . British Journal of Experimental Pathology 1929;10:226-236
2. Abraham EP and Chain E. An enzyme from bacteria able to destroy penicillin. Nature 1940;146:837
3. Kunin CM. Resistance to antimicrobial drugs-a worldwide calamity. Annals of internal medicine. 1993;118(7):557-61.
4. O'Neill J. TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS. THE REVIEW ON ANTIMICROBIAL RESISTANCE . May 2016.
5. de Kraker ME, Stewardson AJ, Harbarth S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS medicine. 2016;13(11):e1002184.
6. Canton R, Coque TM. The CTX-M beta-lactamase pandemic. Current opinion in microbiology. 2006;9(5):466-75.
7. Canton R, Gonzalez-Alba JM, Galan JC. CTX-M Enzymes: Origin and Diffusion. Frontiers in microbiology. 2012;3:110.
8. 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.
9. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. Emerging infectious diseases. 2011;17(10):1791-8.
10. Matsumura Y, Peirano G, Motyl MR, Adams MD, Chen L, Kreiswirth B, et al. Global Molecular Epidemiology of IMP-Producing Enterobacteriaceae. Antimicrobial agents and chemotherapy. 2017;61(4).
11. Carattoli A. Resistance plasmid families in Enterobacteriaceae. Antimicrobial agents and chemotherapy. 2009;53(6):2227-38.
12. Rodriguez-Martinez JM, Machuca J, Cano ME, Calvo J, Martinez-Martinez L, Pascual A. Plasmid-mediated quinolone resistance: Two decades on. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. 2016;29:13-29.
13. 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.
14. Beyrouthy R, Robin F, Lessene A, Lacombat I, Dortet L, Naas T, et al. MCR-1 and OXA-48 In Vivo Acquisition in KPC-Producing Escherichia coli after Colistin Treatment. Antimicrobial agents and chemotherapy. 2017;61(8).
15. WORLD ECONOMIC FORUM. Antimicrobial Resistance Tackling the Gap in R&D Resources with Pull Incentives, May 2018.
16. Lundborg CS, Tamhankar AJ. Antibiotic residues in the environment of South East Asia. BMJ (Clinical research ed). 2017;358:j2440.
17. Holloway KA, Kotwani A, Batmanabane G, Puri M, Tisocki K. Antibiotic use in South East Asia and policies to promote appropriate use: reports from country situational analyses. BMJ (Clinical research ed). 2017;358:j2291.
18. Hara H, Yusaimi YA, Zulkeflle SNM, Sugiura N, Iwamoto K, Goto M, et al. Molecular characterization of multi-drug resistant Escherichia coli isolates from tropical environments in Southeast Asia. The Journal of general and applied microbiology. 2019;64(6):284-92.
19. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta-lactamase-Producing Enterobacteriaceae and Risk Factors Among Healthy Indi-

viduals: A Systematic Review and Metaanalysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2016;63(3):310-8.

20. von Wintersdorff CJ, Penders J, Stobberingh EE, Oude Lashof AM, Hoebe CJ, Savelkoul PH, et al. High rates of antimicrobial drug resistance gene acquisition after international travel, The Netherlands. Emerging infectious diseases. 2014;20(4):649-57.
21. Canton R, Ruiz-Garbajosa P. Co-resistance: an opportunity for the bacteria and resistance genes. Current opinion in pharmacology. 2011;11(5):477-85.
22. 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.
23. UNWTO Tourism Highlights 2018 Edition.
24. de Greeff SCaM, J.W. NethMap 2018 Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands in 2017.
25. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. The Lancet Infectious diseases. 2008;8(3):159-66.
26. Ben-Ami R, Rodriguez-Bano J, Arslan H, Pitout JD, Quentin C, Calbo ES, et al. A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;49(5):682-90.
27. 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.
28. 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.
29. Ben-Ami R, Schwaber MJ, Navon-Venezia S, Schwartz D, Giladi M, Chmelnitsky I, et al. Influx of extended-spectrum beta-lactamase-producing enterobacteriaceae into the hospital. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2006;42(7):925-34.
30. Reddy P, Malczynski M, Obias A, Reiner S, Jin N, Huang J, et al. Screening for extended-spectrum beta-lactamase-producing Enterobacteriaceae among high-risk patients and rates of subsequent bacteremia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2007;45(7):846-52.
31. 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.
32. 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.
33. 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.

34. Lonchel CM, Meex C, Gangoue-Pieboji J, Boreux R, Assoumou MC, Melin P, et al. Proportion of extended-spectrum ss-lactamase-producing Enterobacteriaceae in community setting in Ngaoundere, Cameroon. BMC infectious diseases. 2012;12:53.
35. Luvsansharav UO, Hirai I, Niki M, Nakata A, Yoshinaga A, Moriyama T, et al. Prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae among healthy adult people in Japan. Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy. 2011;17(5):722-5.
36. 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.
37. Nicolas-Chanoine MH, Gruson C, Bialek-Davenet S, Bertrand X, Thomas-Jean F, Bert F, et al. 10-Fold increase (2006-11) in the rate of healthy subjects with extended-spectrum beta-lactamase-producing Escherichia coli faecal carriage in a Parisian check-up centre. The Journal of antimicrobial chemotherapy. 2013;68(3):562-8.
38. 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.