Antibiotic resistant *Escherichia coli*: from livestock animal to food product

Jorrit Kloetstra

J. Kloetstra
S2489058
Rijksuniversiteit Groningen
November 2016
Bachelor Thesis Immunology and Infectious Diseases
Supervisor: Silvia Garcia Cobos

Table of Contents

Abstract

Nowadays, antibiotic resistant bacteria are responsible for a large amount of bacterial infections. These infections are – due to the lack of effective antibiotics – difficult to treat and are therefore a hazard to patients, especially those with a compromised immune system.

The family of *Enterobacteriaceae* is very common in the human and animal gut microbiome. In livestock farming, antibiotics are largely used to prevent infections and because of its beneficial effects on the growth of animals. As a result, several antibiotic resistance mechanisms originated in *Enterobacteriaceae*, including Extended Spectrum beta-lactamases (ESBLs), AmpC beta-lactamases and carbapenemases, all able to hydrolyse beta-lactam antibiotics. Therefore, resistant bacteria are present in the gut microbiome of livestock animals.

These gut bacteria could contaminate the meat products during slaughter of the animals. Also, manure of animals is often used as a fertilizer for vegetables, allowing contamination of the vegetables as well. Since the contaminated meat products and vegetables are consumed by humans, it is possible that the human gut microbiome could be affected, possibly influencing the human health. Therefore, the impact of the presence of antibiotic resistant *Escherichia coli* in the gut microbiome of livestock animals on the food products derived from these animals was examined in this thesis.

Resistance data on Dutch broiler chickens, pigs, and calves and on chicken meat, pork, veal and beef were investigated and compared to each other. Furthermore, data from another European country – Denmark – was examined as well in order to compare the two countries. After comparison, it was not possible to formulate one clear conclusion. The rates of resistances and prevalence of ESBL and AmpC in *E. coli* differ – sometimes greatly – between the animal and its meat product. Several possible causes for these differences exist. In some cases, the sample sizes were too low, causing possible misinterpretations of the data. Furthermore, the meat samples were not taken from the same animals as the studied faecal samples. Also, it is possible samples were taken from imported meat (while faecal sampling was only done from Dutch animals). At last, contamination in slaughterhouses within and between animal species could influence the results from testing of meat samples.

In order to avoid these problems, a study should be performed wherein the gut microbiome of livestock animals is studied by taking faecal samples. After careful slaughter (keeping the cross-contamination as low as possible), meat samples can be taken of the same animals. In such a study, the results are not affected by the fact that the meat samples and microbiome samples are not from the same animal. However, no similar study has been performed yet.

Furthermore, the resistance levels in meat products and animals differ between the Netherlands and Denmark, there are however also similarities. This demonstrates that the different policies in veterinary antibiotic usage in the countries influence the resistance levels in *E. coli* in the animals and the meat products. Changing the laws and rules around the antibiotic administration could result in a positive effect on the health of the society.

Introduction

Nowadays, an increasing part of bacterial infections is caused by antibiotic resistant bacteria. These infections are difficult to treat due to the lack of effective antibiotics and are therefore a hazard to patients, especially those with a compromised immune system. In the United States alone, each year, 23 thousand people die as a consequence of an infection caused by an antibiotic resistant bacteria (CDC Office of Infectious Diseases, 2013). This demonstrates the extent of the problem of antibiotic resistance.

The development of resistance genes can occur in the gut of humans, though as well in the intestinal tract of animals. In livestock farming, antibiotics are greatly used to prevent infections and because of its beneficial effects on the growth of animals. However, because of the great amount of administered antibiotics combined with the rapid evolution in bacteria, multiple antibiotic resistance mechanisms – like AmpC beta-lactamases and Extended-Spectrum beta lactamases (ESBL) – have developed in different bacterial species in the animal gut microbiome. Due to the horizontal and vertical gene transfer between bacteria, antibiotic resistance genes (ARGs) are dispersed between and within bacterial species (Butaye, Devriese, & Haesebrouck, 2003).

As shown in Figure 1, resistant bacteria could be introduced into the human gut microbiome through the consumption of food products contaminated with resistant bacteria. Here, they can exchange genetic material, like ARGs, with the present bacteria in the human gut microbiome and possibly alter the resistance of the human gut microbiome for antibiotics. This could influence the effect of treatment with antibiotics and the human health (Ewers, Bethe, Semmler, Guenther, & Wieler, 2012; Salyers, Moon, & Schlesinger, 2007).

Due to the limited prescription of antibiotics in the Netherlands, the prevalence of resistant bacteria in humans is low. However, the antibiotic use in livestock farming is one of the highest, creating a reservoir with resistant bacteria in livestock animals (Vandenbroucke-Grauls, 2014). Consequently, the presence of antibiotic resistant bacterial species in raw meat is high, as well in fresh vegetables since the – antibiotic resistant bacteria containing – manure of animals is used as fertilizer. This occurs especially in raw chicken meat, but also in other types of meat. A common resistant bacterial species in food – primarily in meat – is *Escherichia coli*. Besides *E. coli*, other *Enterobacteriaceae* are present in food products as well (Rasheed, Thajuddin, Ahamed, Teklemariam, & Jamil, 2014).

This raises the question: What is the impact of the presence of antibiotic resistant *Escherichia coli* in the gut microbiome of livestock animals on the food products derived from these animals?

In order to answer this question, the different resistances to which types of antibiotics in *E. coli* will be specified. Next, the prevalence of resistant *E. coli* in Dutch chickens, cows and pigs will be described. This will be followed by the prevalence of resistant *E. coli* in the meat

products derived from previously mentioned animals. In order to put things in perspective, the data will be compared to another European country – Denmark. At last, a conclusion will be established based on a comparison of the discussed information.

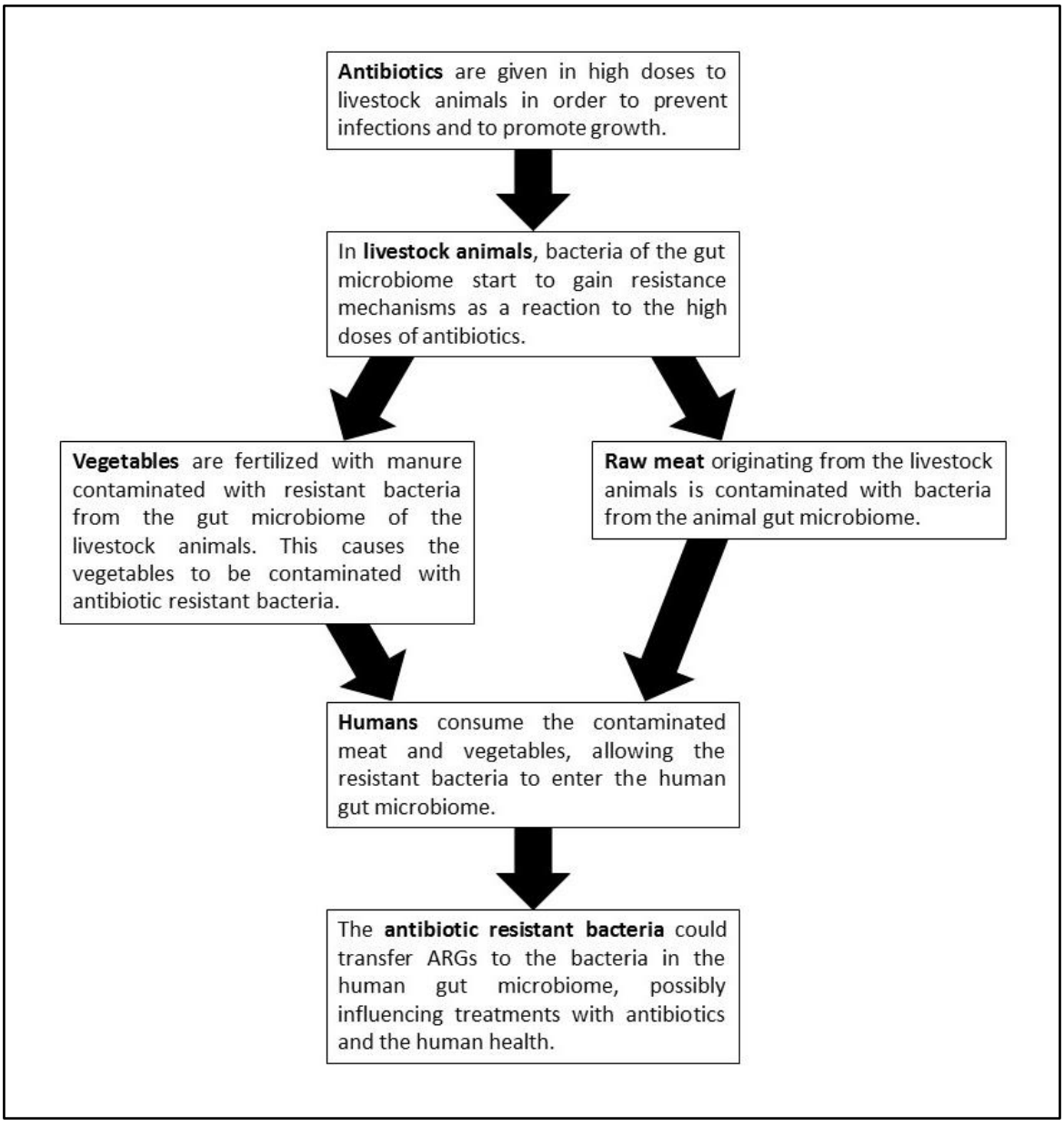

Figure 1: The origination of ARGs in the gut microbiome of livestock animals leads to the contamination of vegetables and raw meat with the resistant bacteria. These contaminated food products are eaten by humans, allowing the resistant bacteria to possibly exchange ARGs with the bacteria in the human gut.

Chapter 1 – Antibiotic resistance mechanisms in the *Enterobacteriaceae*

In 2014, 48 tonnes out of 207 tonnes antibiotics used to treat animals in the Netherlands, consisted of beta-lactam antibiotics (Mevius et al., 2015). This class of antibiotics has been used since the first half of the twentieth century and is still widely used these days. Many antibiotics, like penicillins and cephalosporins, derive from this class (Qin, Panunzio, & Biondi, 2014).

The name stems from the fact that these antibiotics comprise – amongst other things – of a beta-lactam ring. These antibiotics function by inhibiting the synthesis of a cell wall compound, namely peptidoglycan. The final assembly step in the production of peptidoglycan consists of removing the D-alanine terminus from the NAG/NAM-peptide by a Penicillin Binding Protein (PBP). Beta-lactam antibiotics have similar properties compared to this D-alanine terminus. The antibiotic binds to the PBP's active site, however, the beta-lactam ring creates an irreversible bond. By this mechanism, the final step of the peptidoglycan synthesis is disrupted, making it impossible to form a cell wall. Consequently, the bacteria cannot live further. Since peptidoglycan is present in cell walls both from Gram-positive and Gram-negative bacteria, beta-lactam antibiotics have been used to treat a large number of several bacterial infections (Fisher, Meroueh, & Mobashery, 2005).

The rapid evolution of bacterial species allowed them to develop numerous manners to cope with beta-lactam antibiotics disrupting the synthesis of necessary elements in order to survive. In the *Enterobacteriaceae* family, the beta-lactamases originated. This group of enzymes catalyse the hydrolysis of the beta-lactam ring, resulting in an inactivated antibiotic molecule and so, providing a resistance mechanism (Fisher et al., 2005).

Gene transfer between bacteria

The genes responsible for these resistance mechanisms are called antibiotic resistance genes. In presence of antibiotics, primarily the bacteria with these genes continue to live on and reproduce. This occurs to all the bacteria that are exposed to antibiotics. This includes the gut microbiome, which primarily contains members of the *Enterobacteriaceae* family (Bennett, 2008).

In bacteria, genetic information can be transferred both vertical and horizontal – or lateral. With vertical transfer, cell division causes DNA to be copied and divided amongst the two daughter cells. Horizontal transfer of genes can occur in four ways, where genetic information is being transferred from one bacterium to another without cell division. The antibiotic resistance genes are mainly located on conjugative elements: mobile strings of DNA – for example plasmids or transposons – able to be horizontally transferred with less efforts than regular bacterial DNA. For the event of using cell-cell contact to exchange these elements between bacteria, the term conjugation is being used. In the other three cases, cell-cell con-

tact is not required. The uptake and expression of free extracellular DNA is called transformation. As third, transduction occurs when a bacteriophage transfers bacterial DNA instead of bacteriophage DNA to another bacterium. Lastly, Gene Transfer Agents are particles produced by the bacteria and are similar to bacteriophages. These particles are able to transfer parts of the DNA of the cell to other bacteria, achieving horizontal gene transfer (Salyers et al., 2007; von Wintersdorff et al., 2016).

Over the years, various beta-lactam antibiotics have been developed, and with those, various resistance beta-lactamases. These days, the three most important beta-lactamases in *Enterobacteriaceae* are AmpC beta-lactamases, carbapenemases and ESBLs (Mevius et al., 2015).

Extended Spectrum beta-lactamases

This family of beta-lactamases is able to hydrolyse first, second and third generation cephalosporins, as well as penicillins and monobactams. Multiple groups exist within the ESBLs, of whom TEM beta-lactamases, SHV beta-lactamases, CTX-M beta-lactamases and OXA beta-lactamases are the largest. Beta-lactamase inhibitors – like clavulanic acid and tazobactam – are able to inhibit ESBLs. The genes responsible for ESBLs are usually located on plasmids, making exchange of this genetic material occur easily (Ghafourian, Sadeghifard, Soheili, & Sekawi, 2015; Paterson, 2006).

The TEM beta-lactamases are a group of ESBLs that are very common in *E. coli* and *Klebsiella pneumoniae*, however, they are not absent in other species of *Enterobacteriaceae* – like *Salmonella* spp. and *Enterobacter cloacae* – either. The origin of TEM beta-lactamases started with TEM-1. This beta-lactamase was capable of hydrolysing penicillin and first and second generation cephalosporins and is still responsible for 90 percent of the resistance to ampicillin in *E. coli*. Due to a single point mutation with a different amino acid as a consequence, TEM-2 originated. A few years later, TEM-3 was discovered as well. Over time, mutations led to several dozen types of TEM beta-lactamases. These beta-lactamases were able to hydrolyse third generation cephalosporins and monobactams in addition to before – the so-called ESBLs (Bradford, 2001; CDC Office of Infectious Diseases, 2013; Ghafourian et al., 2015).

Similarly to TEM, SHV beta-lactamases developed over time. The first time a SHV beta-lactamase was encountered – SHV-1 beta-lactamase – was in *K. pneumoniae*. In this species, SHV-1 is still in charge of 20 percent of the resistance to ampicillin. Eventually, due to point mutations, the SHV beta-lactamases are expanded to several different SHV beta-lactamases with the same abilities as the different TEM beta-lactamases and are widely present in the *Enterobacteriaceae* (Bradford, 2001; Ghafourian et al., 2015).

The CTX-M beta-lactamases are less similar to the previous two beta-lactamase groups. This resistance mechanism was first encountered in the plasmid of *Kluyvera* spp. The name was derived from the ability to hydrolyse cefotaxime in a greater rate compared to other beta-lactam antibiotics. The plasmid of the *Kluyvera* spp. has been spread over multiple species. For this reason, CTX-M beta-lactamases are mainly encountered in all sorts of species from the *Enterobacteriaceae* family, especially in *S. enterica* and *E. coli* (Bradford, 2001; Ghafourian et al., 2015; Paterson, 2006).

Besides this, the OXA beta-lactamases exist. This group is less represented in the *Enterobacteriaceae*, but is still worth mentioning. OXA beta-lactamases are among class D according to the Ambler categories. These enzymes distinguish themselves from the other ESBLs with their high hydrolytic activity against oxacillin and cloxacillin. Besides this, they are poorly inhibited by clavulanic acid. OXA beta-lactamases are mainly encountered in species from other families than *Enterobacteriaceae* (Bradford, 2001).

AmpC beta-lactamases

Antibiotic resistance was encountered for the first time in 1940 in *E. coli*. Even though initially not identified in that manner, it was actually an AmpC beta-lactamase accountable for the discovered resistance. This resistance mechanism is present in various bacterial species, both Gram-positive and negative, however the most abundant presence is in the *Enterobacteriaceae*.

This subgroup of beta-lactamases (class C according to the Ambler classification) is known for its high affinity for cephalosporins and the ability not to be inhibited by beta-lactamase inhibitors such as clavulanic acid. Besides cephalosporins, AmpC beta-lactamases are able to hydrolyse other antibiotics, e.g. penicillins and monobactams (Abraham & Chain, 1988; Jacoby, 2009).

The AmpC-gene can be located on the plasmid or the chromosome. Plasmid-mediated AmpC allows easy transfer of this gene, causing a faster distribution of antibiotic resistance among bacteria then chromosomal-mediated AmpC. Several species of the *Enterobacteriaceae* family do not possess the chromosomal gene for AmpC, for example *Klebsiella pneumoniae*, *Salmonella* spp., *Citrobacter* spp. and many more. However, these species are able to produce AmpC beta-lactamases as a consequence of having the AmpC gene on a plasmid. Besides this, some species of the *Enterobacteriaceae* – like *E. coli* and *Enterobacter* spp. – have been seen with the AmpC gene both chromosomal of plasmid-mediated (Jacoby, 2009).

Furthermore, there is a difference between inducible AmpC expression and constitutive AmpC expression. Inducible AmpC expression can be triggered by exposure to beta-lactam. On the other hand, constitutive AmpC expression means that the bacteria are continuously producing AmpC beta-lactamases without any trigger being present. In most cases, inducible AmpC expression is present in *Enterobacteriaceae*, however, the constitutive expression of AmpC does occur regularly (Walther-Rasmussen & Hoiby, 2002).

Carbapenemases

Carbapenems are a group of beta-lactam antibiotics whose molecular structure differs from the regular beta-lactam antibiotics. For this reason, they are not hydrolysed by ESBLs or AmpC beta-lactamases, but by the less common carbapenemases. This group of beta-lactamases is a collection of different types of enzymes and therefore are not part of one Ambler class, but of several. The carbapenemases in *Enterobacteriaceae* are found less frequently than ESBLs and AmpC beta-lactamases: only 2 percent of the *Enterobacteriaceae*. Therefore, carbapenems are used as a last option for treating infections. In order to

keep the rate of carbapenems resistance low, these antibiotics are used exclusively for human treatment.
Like mentioned before, carbapenemases are a group of enzymes capable of hydrolysing carbapenems. However, they also possess the ability to react with virtually all other beta-lactam antibiotics. Besides this, beta-lactamase inhibitors – such as clavulanic acid – are not able to reduce the activity of most carbapenemases. Therefore, carbapenemases form a serious problem in the fight against antibiotic resistance, since there are very few resources available to treat infections with carbapenemases-positive bacteria (Mevius et al., 2015; Poirel, Potron, & Nordmann, 2012; Queenan & Bush, 2007).

There are three groups of the carbapenemases with high clinical importance: the KPC group, the metallo-beta-lactamases and the OXA-type carbapenemases. The KPC group (Ambler class A) – an abbreviation for *Klebsiella pneumoniae* carbapenemases – is one of the most effective carbapenemases. KPCs contain the ability to hydrolyse all types of beta-lactam antibiotics. Furthermore, the gene responsible for KPCs is located on the plasmid, allowing for an easy transfer between bacteria resulting in a fast spread. Consequently, this type of carbapenemases leads to problems in treating infections caused by bacteria with KPC carbapenemases in hospitals (Queenan & Bush, 2007).

The second type is the group of metallo-beta-lactamases (MBL) with carbapenemase-characteristics (Ambler class B). These carbapenemases are resistant to all commercially available beta-lactamase inhibitors. Furthermore, its ability to interact with cephalosporins and penicillins stands out. However, it does not react with the monobactam aztreonam. MBLs hydrolyse beta-lactams by interacting through its zinc ions, explaining the “metallo-“ in its name. MLB-genes are present both in the chromosome as in integrons incorporated in gene cassettes. The last allows easy transfer of genes responsible for these beta-lactamases between bacteria (Queenan & Bush, 2007).

The third group is the OXA-type carbapenemases (Ambler class D). This group is named after its ability to hydrolyse oxacillin. Even though OXA-carbapenemases hydrolyse carbapenems not as thorough as the KPCs or MBLs, they are due to their increasing worldwide occurrence of great importance. Especially OXA-48-type carbapenemases – one of the subtypes – is nowadays of great concern. The gene encoding OXA-48 is present on the plasmid, allowing for rapid transfer between bacteria. Since OXA-48 was discovered in *K. pneumoniae* during an outbreak in 2001, this type of carbapenemase has increasingly been involved in outbreaks caused by species of the *Enterobacteriaceae* family in primarily Turkey, the Middle East and North Africa. Furthermore, OXA-48 producers have spread through countries in Europa, causing problems in hospitals due to difficult treatment (Poirel et al., 2012; Queenan & Bush, 2007).

Beta-lactamase inhibitors

In order to increase the effectiveness of beta-lactam antibiotics, one could reduce the activity of beta-lactamases. This is possible by using beta-lactamase inhibitors. The inhibition of beta-lactamases can be achieved by either steric hindering the active site – both reversible and irreversible – or by chemically reacting with the active site of the enzyme, making the enzyme inactive. The last mentioned inhibitors are the so called ‘suicide inhibitors’.

The first clinically used beta-lactamase inhibitor is clavulanic acid. Four decades ago, this product was found not to be a good antimicrobial agent on itself. However, when combined with a beta-lactam antibiotic in *Enterobacteriaceae* among others, the resistance to this antibiotic was significantly reduced. After more research, clavulanic acid was found to be a suicide inhibitor. Where this agent was isolated from the bacterium *Streptomyces clavuligerus*¸ two other suicide inhibitors – sulbactam and tazobactam – were synthesized based on the molecular structure of penicillin. These so called beta-lactam-based beta-lactamase inhibitors – since their structure contains a beta-lactam ring – are all clinically used in combination with antibiotics. Due to this use, resistance to these inhibitors originated in ESBLs, as well in AmpC beta-lactamases and carbapenemases (Drawz & Bonomo, 2010).

Also, a new inhibitor – avibactam – has been developed and is now clinically used to treat infections. This synthetic non-beta-lactam-based beta-lactamase inhibitor is being used in combination with ceftazidime – a third generation cephalosporin antibiotic – and is highly effective against mostly all *Enterobacteriaceae* harbouring beta-lactamases (Papp-Wallace & Bonomo, 2016).

Chapter 2 – Antibiotic resistant *Escherichia coli* in livestock animals

The *Enterobacteriaceae* are a family of Gram-negative bacterial species. Among those species are for example *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter* spp. These species are the cause of common infections like pneumonia and urinary tract infection. Besides this, these bacteria are worldwide largely present in the gut microbiome of humans and animals and therefore, play a role in the health of animals and humans. In this chapter, the focus will be on the presence of antibiotic resistant *E. coli* in the gut microbiome of several livestock animals (Denton, 2007; Paterson, 2006).

The total amount of sold antibiotics for veterinary purposes in the Netherlands peaked in 2007 at 565 tons in total. Starting that year, it has drastically decreased to 217 tons in 2013. From then on, the amount roughly stabilised: in 2014, the amount had decreased with merely ten tons and in 2015, the decrease was only with one ton. However, the sales of beta-lactam antibiotics started decreasing not earlier then in 2009 until 2013, and increased even slightly in 2014, consisting of 48 tons. In 2015, this quantity was estimated at 45 tons beta-lactams. Therefore, the beta-lactams comprise currently almost a quarter of the sold antibiotics in the Netherlands. Consequently, they have a large impact on the antibiotic resistance on the animal gut microbiome (Mevius et al., 2015; Veldman et al., 2016).

Broiler chickens

This breed of chickens is specifically raised for meat consumption, in contrast to the egg-laying hens. According to the screenings for the year 2015 published in the MARAN report, high doses of antibiotics were administered to these broiler chickens. The MARAN (Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands) report is a annually published compilation of data about the affairs about and around antibiotic resistance in animals in the Netherlands. This study is performed by the Central Veterinary Institute of Wageningen University and Research Centre in collaboration with the Food and Consumer Product Safety Authority, the National Institute for Public Health and the Environment and the Netherlands Veterinary Medicines Authority. To broiler chickens, penicillins were given in particular: 8.44 $DDDA_{NAT}$ (*Defined Daily Doses Animals* – a unit based on the total amount of administered drug for the whole animal sector and the weight of that particular sector. This allows easy comparison between different types of animal sectors (Veldman et al., 2016)). To put this in perspective: according to these calculations, broiler chickens received – relatively – several times more penicillins in comparison to cattle (1.26 $DDDA_{NAT}$) and pigs (1.93 $DDDA_{NAT}$) (Veldman et al., 2016).

In the Netherlands, the gut microbiome of broiler chickens has been tested for antibiotic resistance in several manners for the year 2015. When testing the *E. coli* from this gut microbiome, it came forward that 53.3 percent of the faecal samples were resistant to the beta-lactam antibiotic ampicillin. Furthermore, a few of the *E. coli* isolates were also resistant to

the third generation cephalosporins cefotaxime (2.5 percent) and ceftazidime (2.5 percent). Additionally, there appeared to be no resistance to meropenem – a carbapenem – implicating an absence of carbapenemases in the gut microbiome of broiler chickens (Figure 2.1A). Nevertheless, the resistance to the former three beta-lactams implicates the presence of ESBLs or AmpC beta-lactamases in the gut bacteria (Veldman et al., 2016).

The *E. coli* isolates were tested for the presence of ESBL and AmpC. Out of these tests, 56.5 percent of the faecal samples came forward as positive for possessing *E. coli* with either an ESBL or AmpC gene. Within these positive results, 96.2 percent tested positive for an ESBL gene and 3.8 percent was positive for an AmpC gene. With other words, of all tested samples, 54.3 percent possessed *E. coli* with an ESBL gene and 2.2 with an AmpC gene (Veldman et al., 2016). This can be seen in Figure 2.1B.

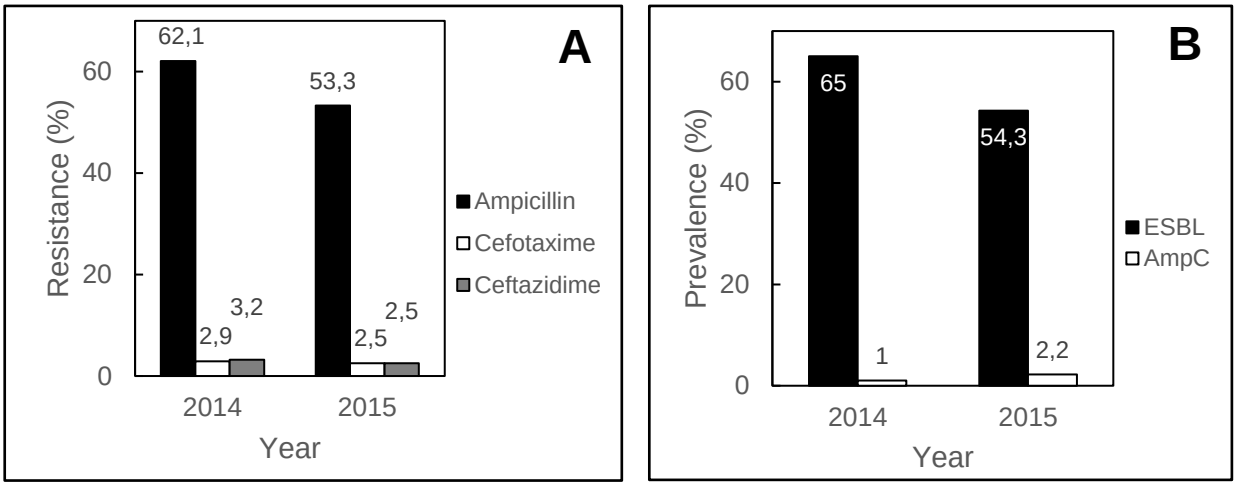

Figure 2.1: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples originating from the gut microbiome of Dutch broiler chickens in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples originating from the gut microbiome of Dutch broiler chickens in 2014 and 2015 (**B**).

While these resistance rates are very high, there is actually a decrease compared to the year before. In the MARAN 2014 report, the resistance rates to ampicillin, cefotaxime and ceftazidime in *E. coli* isolates from the gut microbiome of broiler chickens was higher than in 2015 (respectively 62.1, 2.9 and 3.2 percent – the resistance rate to meropenem was also zero percent, implicating no presence of carbapenemases), as can be seen in Figure 2.1A. However, when focusing at the prevalence of ESBL and AmpC genes, 65 percent of the samples was positive for ESBL possessing *E. coli*, while only 1 percent was positive for AmpC. This all comes down to a decrease of the resistance rates and ESBL prevalence from 2014 to 2015, but an increase of 1.2 percent in the AmpC prevalence in the gut microbiome of broiler chickens (Mevius et al., 2015; Veldman et al., 2016). (Figure 2.1B).

Pigs

The dose of antibiotics administered to pigs is not as high as in chickens, however, the resistance in *E. coli* still reaches significant levels in the Netherlands. In the same way antibiotic resistance in the gut microbiome of broilers was studied in the MARAN 2016 report, pigs have been studied in the Netherlands that year. *E. coli* from the faecal samples from the guts

of pigs were tested for resistance to several antibiotics. In 28.9 percent of the cases – approximate half of the levels as in broilers – the samples possessed ampicillin resistant *E. coli*. Furthermore, resistance to the two cephalosporins – cefotaxime and ceftazidime – were both 0.3 percent. Equally as in the chickens, no resistance to meropenem was identified (Figure 2.2A). Based on these results it is possible to say that both ESBLs or AmpC beta-lactamases could be present, however no presence of carbapenemases is expected (since no resistance to carbapenems was found) (Veldman et al., 2016).

The testing of the *E. coli* samples for ESBL and AmpC presence for that year resulted in the following: in total, 12.3 percent of the samples were positive for either possessing an ESBL or AmpC gene. Within these positive samples, 33.9 percent tested positive for an AmpC gene, while the other 66.1 percent possessed an ESBL gene. In conclusion: 8.1 percent of all *E. coli* samples possessed an ESBL gene, while 4.2 percent possessed an AmpC gene (Veldman et al., 2016).

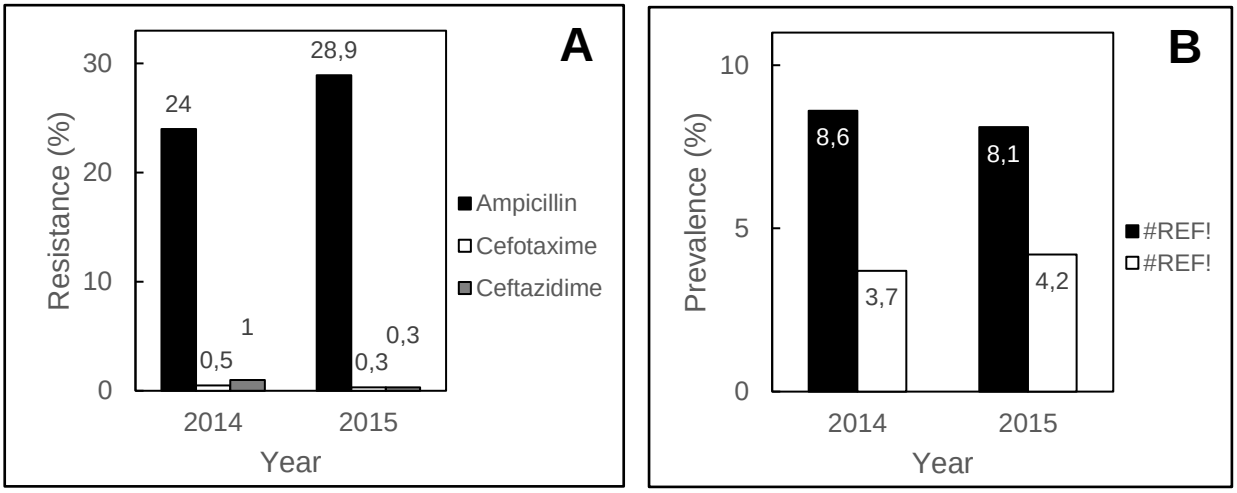

Figure 2.2: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples originating from the gut microbiome of Dutch pigs in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples originating from the gut microbiome of Dutch pigs in 2014 and 2015 (**B**).

In comparison to 2014, resistance to ampicillin increased with 4.9 percent in 2015. However, the resistance to cefotaxime was 0.5 percent in 2014. This decreased to 0.3 percent in 2015. Furthermore, the resistance to ceftazidime decreased as well in 2015, from 1.0 to 0.3 percent. Resistance to meropenem remained zero percent. The total prevalence of ESBL and AmpC genes was established at 12.3 percent both in 2014 as 2015. However, the balance between ESBL and AmpC shifted slightly from 8.6 percent and 3.7 percent respectively in 2014, to 8.1 percent and 4.2 percent in 2015 (Mevius et al., 2015; Veldman et al., 2016). (Figure 2.2).

Calves

In the MARAN report, the gut microbiome of older cattle was not studied. However, faecal samples were taken from veal calves and examined. A distinction was made between white veal calves and rosé veal calves. White veal calves are slaughtered at younger age, while receiving higher doses of antibiotics compared to rose veal calves. Rosé veal calves are

slaughtered at an older age. This causes differences in the prevalence of antibiotic resistance in *E. coli* for the two groups of calves (Veldman et al., 2016).

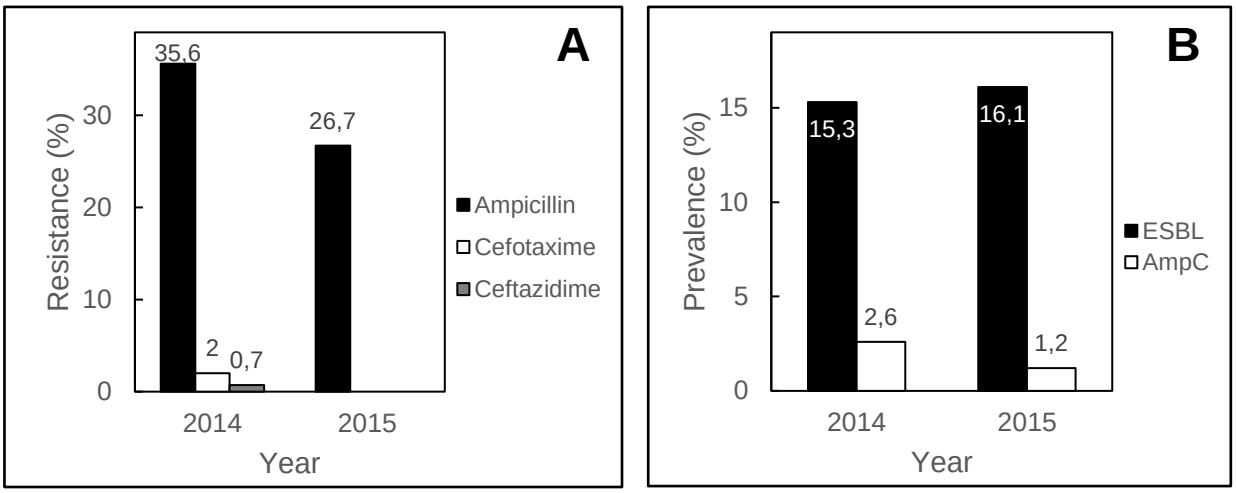

Figure 2.3: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples originating from the gut microbiome of Dutch white veal calves in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples originating from the gut microbiome of these animals in 2014 and 2015 (**B**).

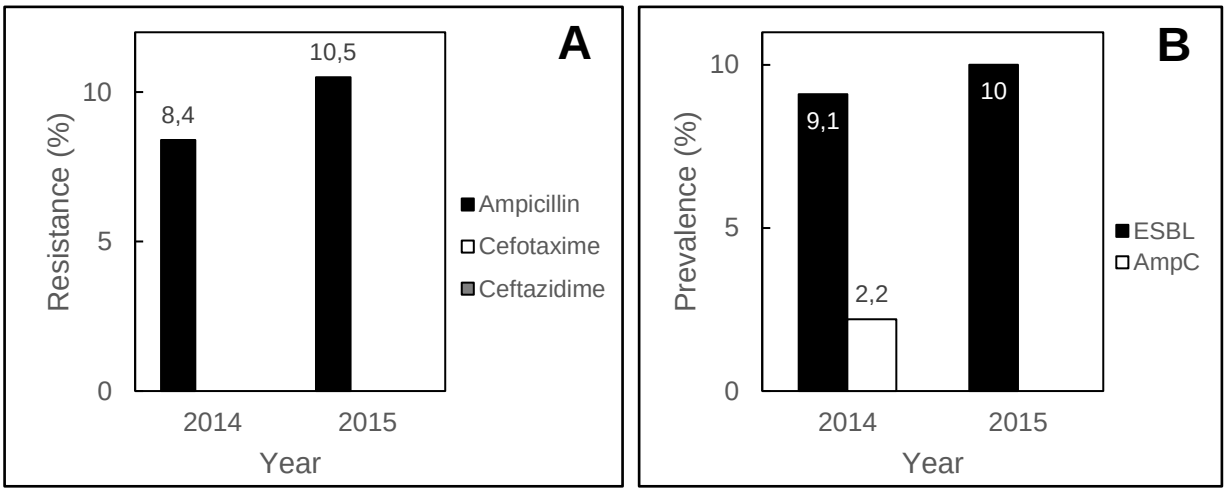

Figure 2.4: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples originating from the gut microbiome of Dutch rosé veal calves in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples originating from the gut microbiome of these calves in 2014 and 2015 (**B**).

In 2015, testing of the faecal samples from white calves for antibiotic resistance in the *E. coli* showed resistance to ampicillin in 26.7 percent of the cases, while merely 10.5 percent of the samples obtained from rosé calves were resistant to ampicillin. Furthermore, no resistance occurred to cefotaxime, ceftazidime and meropenem in both kinds of calves. Testing for ESBL and AmpC presence in the *E. coli* resulted in the following: 17.3 percent of the samples from white calves were positive for either ESBL or AmpC. This 17.3 percent consists of 1.2 percent being accounted for by the AmpC presence and 16.1 percent by the ESBL pres-

ence. ESBL presence in *E. coli* in the gut microbiome of rosé calve was confirmed in 10 percent of the faecal samples, while no presence of AmpC was confirmed (Veldman et al., 2016). (Figure 2.3 and 2.4).
In comparison with 2014, the resistance in *E. coli* from the gut of white calves decreased. Resistance to ampicillin, cefotaxime and ceftazidime was recorded at respectively 35.6 percent, 2.0 percent and 0.7 percent in 2014, while resistance to ampicillin was 26.7 percent and zero resistance to the two cephalosporins in 2015. In rosé veal calves, resistance to ampicillin increased slightly from 8.4 percent in 2014 to 10.5 percent in 2015. Additionally, the resistance in rosé calves to cefotaxime and ceftazidime remained zero (Mevius et al., 2015; Veldman et al., 2016). (Figure 2.3 and 2.4).

The total prevalence of ESBL and AmpC beta-lactamases in white veal calves decreased slightly from 17.9 percent to 17.3 percent in 2015. This consists of a decrease of AmpC presence from 2.6 percent to 1.2 percent in 2015, but an increase of ESBL from 15.3 percent to 16.1 percent. In rosé calves, total prevalence of ESBL and AmpC was identified as 11.3 in 2014. This decreased to 10 percent in 2015. The prevalence of ESBL in 2014 was 9.1 percent, but went up to 10 percent in 2015. AmpC presence decreased from 2.2 percent in 2014 to 0 percent in 2015 (Figure 2.3 and 2.4) (Mevius et al., 2015; Veldman et al., 2016). (Figure 2.3 and 2.4).

An example to contrast with: The Denmark situation

In Denmark, antibiotic resistance in animals is examined by the National Food Institute and the National Veterinary Institute (both at the Technical University of Denmark) and the Statens Serum Institut. This is performed similar to the MARAN report in the Netherlands. The results are published in an annual report – the DANMAP report – and therefore, a good comparison between the Netherlands and another European country (in this case, Denmark) can be made. In the DANMAP report, faecal samples from broiler chickens, cattle and pigs were examined for antibiotic resistant *E. coli*. Likewise, retail meat samples from pork, chicken and beef were tested for *E. coli* and its resistance to antibiotics. Equally to the Netherlands, use of carbapenems and cephalosporins is not allowed in Denmark in veterinary medicine. In general, however, less antibiotics are used in the animal sector in Denmark, resulting in interesting differences, but also similarities (Bager et al., 2015; European Medicines Agency, 2015).

In Danish broiler chickens, the resistance rates in *E. coli* were several times lower compared to Dutch chickens. Resistance to ampicillin was determined at 14 percent in 2014 (while 62.1 percent in the Netherlands that year). Furthermore, cefotaxime resistance was zero percent, equally to ceftazidime resistance (while respectively 2.9 and 3.2 percent in the Netherlands) and meropenem (equal in the Netherlands) (Bager et al., 2015; Mevius et al., 2015).

However, resistant *E. coli* in pigs appears to be more present in Denmark than in the Netherlands. Resistance to ampicillin in *E. coli* was identified in 33 percent of the samples, versus 24.0 percent in the Netherlands. However, no resistance to cephalosporins was found in Denmark, while this did occur in the Netherlands (0.5 percent for cefotaxime and 1.0 percent for ceftazidime). Furthermore, no resistance to meropenem occurred in both countries (Bager et al., 2015; Mevius et al., 2015).

Resistant *E. coli* in cattle was solely occurring to ampicillin in 8.4 percent of the faecal samples in 2014. No resistance was found for cephalosporins or carbapenems. In the MARAN report, only veal calves were tested instead of cattle. Whereas samples obtained from Dutch white veal calves were resistant in a large extent compared to Danish cattle, resistance in rosé veal calves is very similar to Danish cattle: 8 percent resistance to ampicillin, and no resistance for cephalosporins or meropenem (Bager et al., 2015; Mevius et al., 2015).

Chapter 3 – Antibiotic resistant Escherichia coli in retail meat

Besides their presence in livestock animals, *Enterobacteriaceae* are present in retail meat as well. It appears to be that *E. coli* is the most occurring species in meat. In raw chicken meat, the greater part contains *E. coli* according to studies performed by Veldman et al. (2016), Overdevest et al. (2011). Besides chicken meat, presence of *E. coli* was also verified in raw sheep meat (Rasheed et al., 2014), raw beef (Kawamura, Goto, Nakane, & Arakawa, 2014; Ojer-Usoz et al., 2013; Overdevest et al., 2011), raw ground meat (Overdevest et al., 2011) and raw pork meat (Kawamura et al., 2014; Ojer-Usoz et al., 2013; Overdevest et al., 2011). In a few cases, there was also a small presence of other *Enterobacteriaceae*, like *Klebsiella* spp. and *Escherichia fergusonii* (Cohen Stuart et al., 2012; Overdevest et al., 2011).

In this chapter however, the focus will again be on *E. coli*. The meat products that will be discussed are derived from the discussed livestock animals in the previous chapter: chicken meat, pork and beef.

Chicken meat

Raw chicken meat contains by far the most bacteria compared to the other types of meat. However, the amount of the present antibiotic resistant bacteria differs greatly between the researches and countries these studies were performed in, probably caused by the different administered doses of antibiotics (European Medicines Agency, 2015).

For example, a research carried out in Brazil encountered that in 23.3 percent of the examined chicken meat samples antibiotic resistant *E. coli* was present (Rasheed et al., 2014). Meanwhile in the Netherlands, 76.8 percent of the chicken meats used in this research were positive for antibiotic resistant *E. coli* (Overdevest et al., 2011). This is similar in the MARAN report. Here was found that 67 percent of the samples acquired from domestic chicken meat and 84.4 percent of the imported chicken meat were ESBL positive, that means an average of 75.7 percent ESBL positive raw chicken meat samples (Mevius et al., 2015). In Denmark, only 30 percent of the chicken meat from domestic broiler chickens contained antibiotic resistant bacteria, while 76 percent of the imported chicken meat (exporting country unknown) contained antibiotic resistant bacteria. Even approximately 40 percent was multi-resistant (Bager et al., 2015). A study performed in the United Kingdom concluded that only 24 percent of the raw chicken meat contained ESBL positive *E. coli* (Alliance to Save our Antibiotics, 2016).

As can be seen above, imported chicken meat in different countries possesses approximate the same amount of antibiotic resistant bacteria, whereas the samples from domestic chicken meat contain varying amount of resistant bacteria between countries. This corresponds with the idea that the differences could be caused by different antibiotic doses applied between countries.

According to the MARAN report for 2015, 41.8 percent of the *E. coli* found in samples from chicken meat were resistant to ampicillin. Furthermore, resistance to cefotaxime in *E. coli* occurred in 4.3 percent of the samples, while 5 percent of the samples possessed ceftazidime resistant *E. coli*. However, no resistance to meropenem was identified, implying only the possibility of ESBL and AmpC as resistance mechanisms, and not carbapenemases (Veldman et al., 2016). (Figure 3.1A).

The total occurrence of ESBL and AmpC in 2015 was determined at 39.4 percent in *E. coli* acquired from chicken meat. However, after distinguishing between the two resistance mechanisms, no sample appeared to possess an AmpC. In conclusion, from all of the samples from chicken meat, 39.4 percent possessed an ESBL positive *E. coli*. Furthermore, 66.7 percent of the samples from imported chicken meat were positive for ESBL possessing *E. coli* (Veldman et al., 2016). (Figure 3.1B)

In respect of 2014, antibiotic resistance appears to have increased. That year, 40.7 percent of the *E. coli* in the chicken meat samples was resistant to ampicillin, which means a small increase of 1.1 percent in 2015. Furthermore, cefotaxime resistance was determined at 1.9 percent, while this increased to 4.3 percent in 2015. Likewise, the ceftazidime resistance was determined at 3.0 percent, but increased with 2 percent in 2015. The resistance to meropenem however, is equal to the resistance in 2015, namely zero percent. However, in 2014, the total prevalence of ESBL and AmpC was determined at 67 percent in chicken meat, a rather larger number compared to 2015. Approximate 4.4 out of the 67 percent is comprised by AmpC presence, while 62.2 percent is accounted for by ESBLs. This however, corresponds with the decrease in ESBL and AmpC prevalence over the years before according to the MARAN report (data not shown) (Mevius et al., 2015; Veldman et al., 2016). (Figure 3.1).

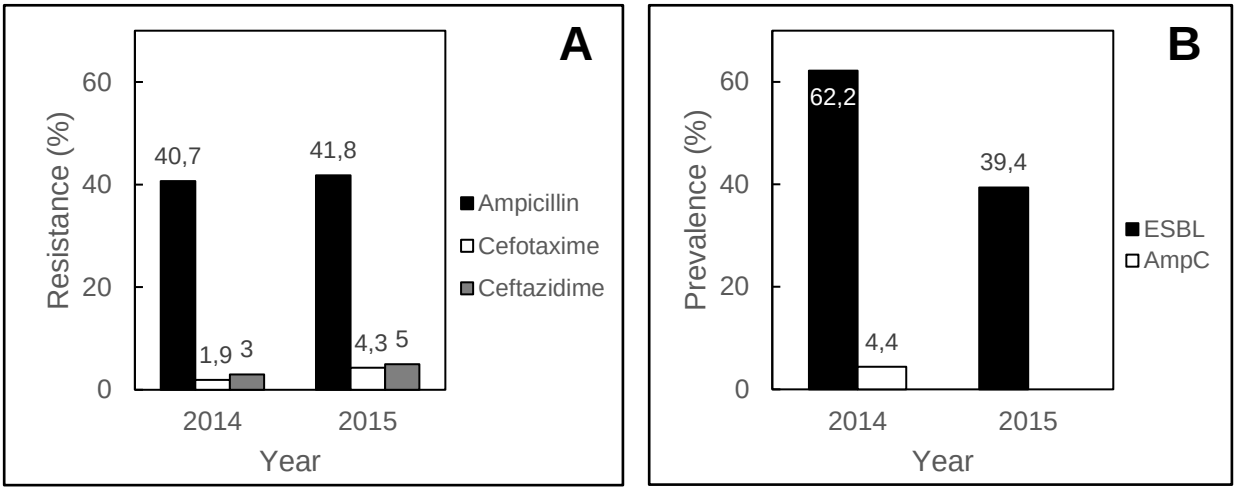

Figure 3.1: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from chicken meat from Dutch retail in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples from chicken meat from Dutch retail in 2014 and 2015 (**B**).

Pork

In comparison to chicken meat, antibiotic resistant *E. coli* are less present in pork meat. According to the MARAN report over the year 2015, resistance to ampicillin is present in 15.1 percent of the *E. coli* samples. Furthermore, cefotaxime resistance and ceftazidime resistance are respectively zero and 1.7 percent. No meropenem resistance was identified, implying the absence of carbapenemases (Veldman et al., 2016). (Figure 3.2A).

The total presence of ESBL and AmpC in *E. coli* comprises 0.8 percent among the pork samples in 2015. This total 0.8 percent consists of 0.6 percent accounted for by ESBL presence and 0.2 percent by AmpC beta-lactamase presence (Veldman et al., 2016). (Figure 3.2B).

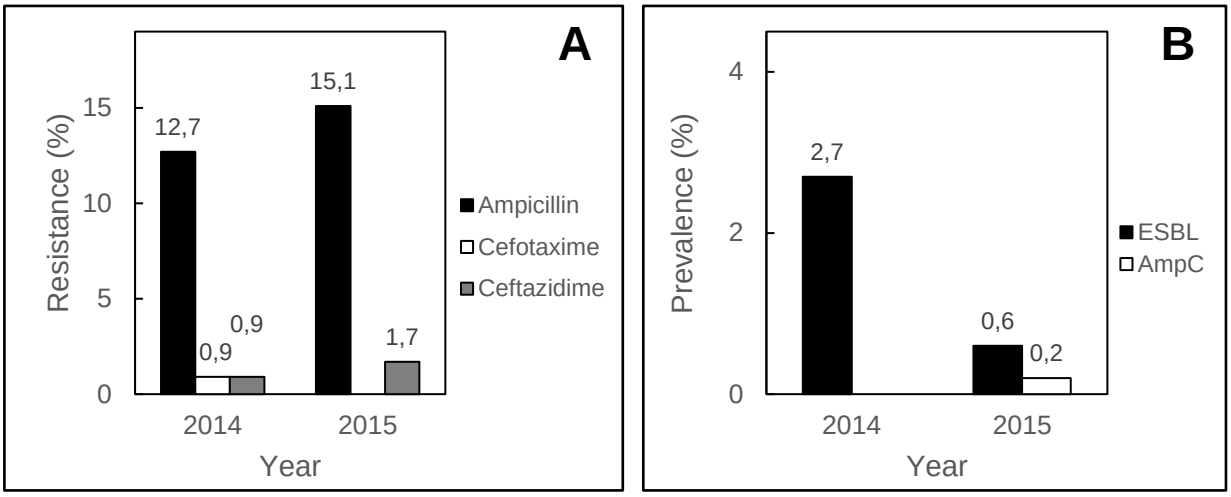

Figure 3.2: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from pork from Dutch retail in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples from pork from Dutch retail in 2014 and 2015 (**B**).

In comparison to 2014, resistance to ampicillin increased from 12.7 percent to 15.1 percent, as well as resistance to ceftazidime from 0.9 percent to 1.7 percent. Resistance to cefotaxime was 0.9 percent in 2014, this however decreased to zero percent in 2015. Equal to 2015, meropenem resistance was not present in the *E. coli* samples. In 2.7 percent of the samples, ESBL was present, while no AmpC beta-lactamase was identified. This ESBL prevalence decreased with 1.9 percent in 2015, while AmpC resistance was identified in 0.6 percent of the samples, a slight increase. In conclusion, the resistance to beta-lactam antibiotics and the prevalence of AmpC beta-lactamases increased slightly, while ESBL prevalence decreased (Mevius et al., 2015; Veldman et al., 2016). (Figure 3.2).

Beef

Whereas chicken meat appears to contain the most resistant *E. coli*, beef appears to contain the least. In the MARAN report for 2015, ampicillin resistance was found to be present in 10.2 percent of the samples. Furthermore, resistance to cefotaxime and ceftazidime were respectively 2.2 and 2.9 percent. Resistance to the carbapenem meropenem was not found in the *E. coli*. This resistance could be caused by both ESBL and AmpC beta-lactamases.

The total prevalence hereof is 1.7 percent. Only 0.1 percent is accounted for by AmpC, the other 1.6 percent of the samples contains an ESBL (Veldman et al., 2016). (Figure 3.3).

The presence of antibiotic resistant *E. coli* seems to have increased in a small manner compared to 2014. Resistance to ampicillin occurred 7.8 percent of the samples, whereas in 2015 this was 10.2 percent. However, resistance to cephalosporins was determined at 1.9 percent for cefotaxime and 2.7 percent for ceftazidime, while this was respectively 2.2 and 2.9 percent in 2015. Nevertheless, presence to the antibiotic resistance increased from 2014 to 2015. Furthermore, total prevalence of ESBL and AmpC has decreased in a small manner. However, in 2015, the occurrence of AmpC beta-lactamases appeared to increase with 0.1 percent, while AmpC did not prevail in 2014 in the *E. coli* samples from beef. The prevalence of ESBL decreased from 2.2 percent in 2014 to 1.6 percent in 2015 (Mevius et al., 2015; Veldman et al., 2016). (Figure 3.3).

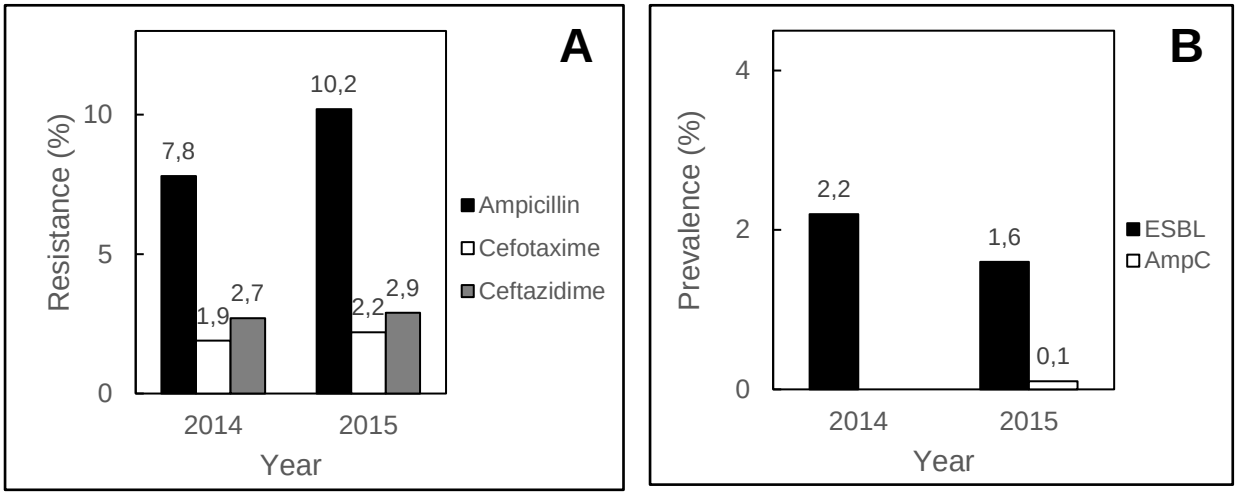

Figure 3.3: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from beef from Dutch retail in 2014 and 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples from beef from Dutch retail in 2014 and 2015 (**B**).

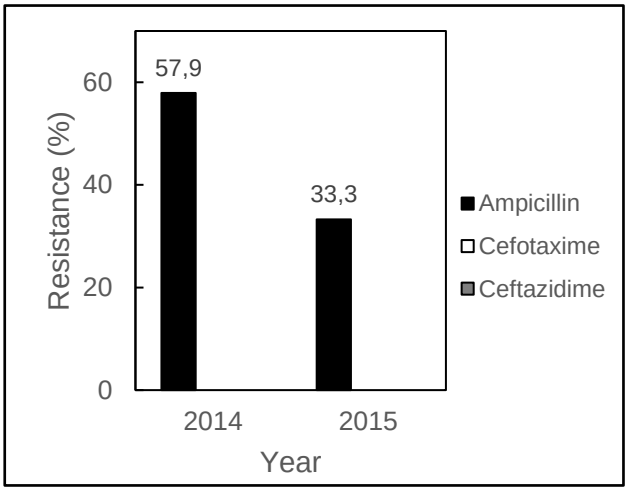

Figure 3.4: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from veal from Dutch retail in 2014 and 2015. Prevalence of ESBL and AmpC has not been included since there have not been specific results in 2014.

Veal however, contains the highest amount of antibiotic resistant *E. coli* along with chicken meat. In 2015, 33.3 percent of the veal samples were resistant to ampicillin. Resistances to the cephalosporins and meropenem were not identified. However, these results are not completely reliable due to their rather small sample size – namely six samples. Furthermore, neither ESBL nor AmpC beta-lactamases were identified in the *E. coli* samples. However, this could be a consequence of the small sample size (Veldman et al., 2016). (Figure 3.4).
In the MARAN report of 2014, a larger sample size – 19 veal samples – was used, even though this still is not reliable. Here, the researchers found the *E. coli* samples to be resistant to 57.9 percent of the cases. Furthermore, no resistance to cefotaxime, ceftazidime and meropenem was identified. Furthermore, in 3.1 percent of the samples, ESBL or AmpC was confirmed. However, the ratio between these two mechanisms has not been specified. Based on these results there seems to be a decrease in the antibiotic resistance in *E. coli* in veal. This however – like mentioned before – could be caused by the unreliably small sample size (Mevius et al., 2015; Veldman et al., 2016).

An example to contrast with: The Denmark situation

As in the previous chapter the Danish and Dutch livestock animals were compared, the meat products from both countries will be compared to each other.

The *E. coli* samples obtained from chicken meat were resistant to ampicillin in 19 percent of the cases. Furthermore, resistance to the cephalosporins (cefotaxime and ceftazidime) was 1 percent for both. In the Netherlands, these resistance rates were much higher (respectively 40.7, 1.9 and 3.0 percent). In both countries however, no resistance to meropenem was determined (Bager et al., 2015; Mevius et al., 2015).

In pork, resistance in *E. coli* to ampicillin appeared in 36 percent of the samples. However, no resistance to cephalosporins or meropenem was found. In the Netherlands, resistance to ampicillin was much less (12.7 percent). However, Dutch *E. coli* samples from pork were resistant to cefotaxime and ceftazidime in both cases with 0.9 percent. Meropenem resistance did not occur in any of the two countries (Bager et al., 2015; Mevius et al., 2015).

Danish beef appears to possess more resistant *E. coli* than in the Netherlands. Resistance to ampicillin is 11 percent (opposed to 7.8 percent in the Netherlands). Furthermore, resistance to cefotaxime was found to be 4 percent and to ceftazidime 2 percent (1.9 and 2.7 percent in the Netherlands). Furthermore, no resistance to carbapenems were found in Danish or Dutch beef samples (Bager et al., 2015; Mevius et al., 2015).

The antibiotic resistance in Danish chicken meat appears to be less than half of the resistance rate in the Netherlands. However, resistant *E. coli* is more present in pork from the Denmark than the Dutch pork. In beef, there merely seem to be minor differences between the two countries.

Chapter 4 – Discussion & Conclusion: from livestock animal to food chain

The comparison

Broiler chickens and chicken meat

Chicken meat contains rather less *E. coli* with resistance to ampicillin than the broiler chickens. However, resistance to both ceftazidime and cefotaxime are higher in *E. coli* from chicken meat. This could be – like the researchers in the MARAN 2016 report stated – caused by unintentionally testing of more or less imported chicken meat, resulting in shifted values of antibiotic resistance. In total, there is less resistance (7.2 percent) to beta-lactams in the chicken meat than in the broiler chickens in 2015 (Veldman et al., 2016). (Figure 4.1).

In comparison to 2014, resistance to ampicillin and cephalosporins decreased in *E. coli* in broiler chickens in 2015. The ampicillin resistance decreased from 62.1 percent to 53.3 percent and the cephalosporin resistance decreased from 6.1 percent to 5 percent. In chicken meat however, while ampicillin resistance stayed nearly the same (in 2014: 40.7 percent; in 2015: 41.8 percent), the resistance to cephalosporins increased in 2015 in chicken meat from 4.9 percent to 9.3 percent. This however, like mentioned above, could be the consequence of unintentionally testing of more or less imported meat. Nevertheless, the difference between resistant *E. coli* in broilers and chicken meat has shrunk due to the decrease in resistance in broiler chickens in 2015 (Mevius et al., 2015; Veldman et al., 2016).

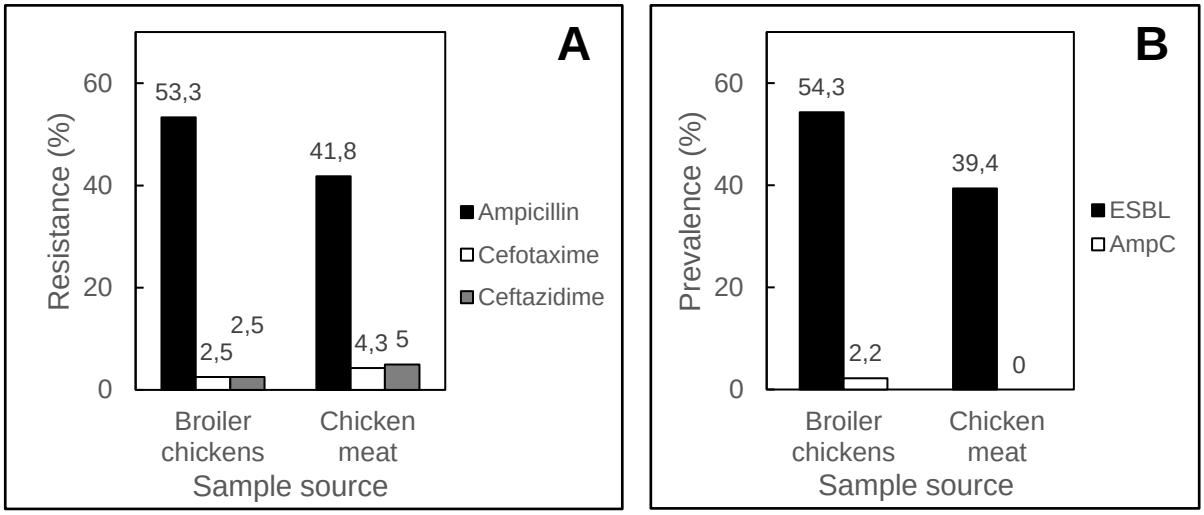

Figure 4.1: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from Dutch broiler chickens and chicken meat from Dutch retail in 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples from these sources in 2015 (**B**).

The ESBL prevalence appears to be approximate 15 percent lower in *E. coli* from chicken meat than from broiler chickens. Moreover, there is no sign of AmpC beta-lactamases in the chicken meat samples, while they comprise 2.2 percent of the *E. coli* samples in broiler chickens. In 2014, prevalence of ESBL in the *E. coli* from broiler chickens, but especially in chicken meat, was much higher than in 2015: in broiler chickens, the prevalence in *E. coli* decreased with 10.5 percent in broiler chickens and even with 22.8 percent in chicken meat. Furthermore, the AmpC beta-lactamases were present in a relatively large amount in chicken meat with 4.4 percent, while this disappeared in 2015 (Veldman et al., 2016). (Figure 4.1).

Overall, beta-lactam resistance in *E. coli* has decreased in 2015 in both broiler chickens and chicken meat, which corresponds with the years before. This could be the consequence of the reduced use of beta-lactams in the veterinary sector starting in 2010. Since that year, the occurrence of resistance to ampicillin and cephalosporins appears to decrease (Veldman et al., 2016).

Pigs and pork

Similar to the broiler chickens and the chicken meat, the ampicillin resistance in *E. coli* from pigs is higher than the ampicillin resistance in pork in (pigs: 28.9 percent; pork: 15.1 percent in 2015). The difference is that in pigs compared to chicken, the resistance is nearly twice as high as the resistance in the pork acquired *E. coli*. It is however lower than in broilers and chicken meat. Furthermore, the resistance to cefotaxime and ceftazidime differs between the *E. coli* from pigs and pork. Ceftazidime resistance appears to be 1.5 percent higher in pork *E. coli* than in pigs, whereas resistance to cefotaxime is not present in pork samples, while comprising 0.3 percent of the samples from pigs (Veldman et al., 2016). (Figure 4.2).

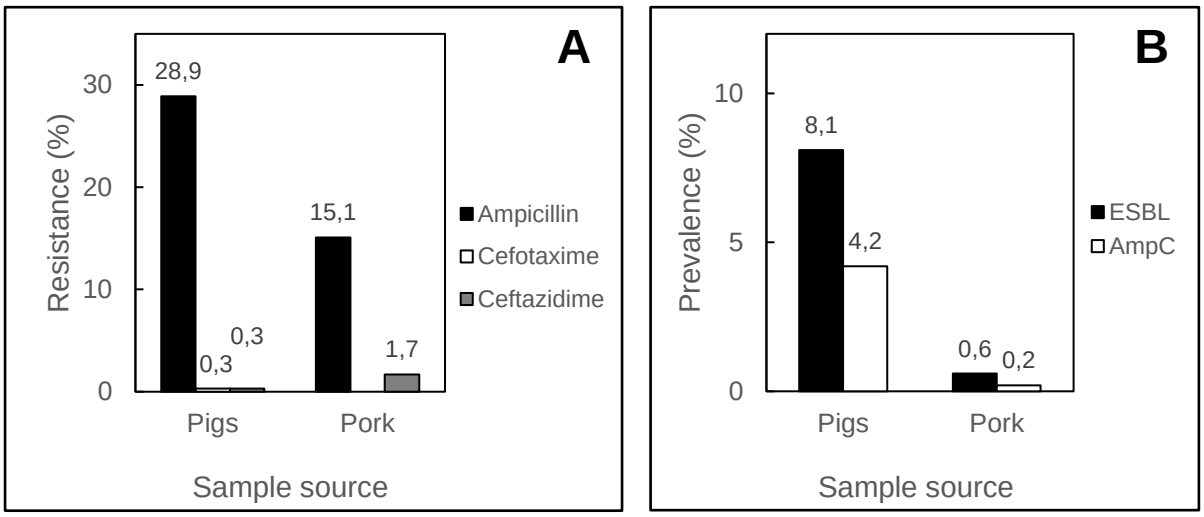

Figure 4.2: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from Dutch pigs and pork from Dutch retail in 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples from these sources in 2015 (**B**).

Similar to 2015, the difference between the resistance in pigs is roughly twice as high as in pork in 2014 (pigs: 24 percent; pork: 12.7 percent). However, the total beta-lactam resistance in *E. coli* increased in 2015, as well in pigs (with 4 percent) as in pork (2.3 percent). The resistance to ampicillin went up in with 4.9 percent pigs and with 2.4 percent in pork, and

ceftazidime resistance in pork increased also with 0.8 percent. However, the resistance to cephalosporins in pigs decreased with 0.9 percent in 2015, as well as resistance to cefotaxime in pork (with 0.9 percent). (Mevius et al., 2015; Veldman et al., 2016).

Furthermore, the prevalence of ESBL and AmpC is over tenfold lower in pork than in pigs in 2015. However, whereas the combined testing for ESBL and AmpC was done with a high sample size, the distinguishing between those two was done with only 9 samples *E. coli* from pork. A sample size of 9 is not high enough to draw strong conclusions. Therefore, it is clear that the total prevalence of ESBL and AmpC is 0.8 percent, but it is not clear how the ratios are distributed within the 0.8 percent. (Veldman et al., 2016). (Figure 4.2).

In 2014, the ESBL prevalence in pork samples was about a quarter (2.2 percent) of the prevalence in pig samples (8.6 percent). In that year, several times more ESBL was found in the pork samples than in 2015 (0.6 percent). Furthermore, no AmpC beta-lactamases were present in the *E. coli* samples from pork, whereas this is present in pigs (3.7 percent). However, the testing for AmpC beta-lactamases was performed with solely a few samples, weakening the conclusions that can be drawn from these results (Mevius et al., 2015; Veldman et al., 2016).

Calves, veal and beef

In samples from white veal calves, the resistance to ampicillin was less present than in veal meat samples (26.7 percent versus 33.3 percent in 2015). Furthermore, in both was no resistance found to both ceftazidime and cefotaxime. However, only six veal samples were used to test the resistance in *E. coli* in veal in 2015, therefore no conclusions about all Dutch veal can be drawn. The resistance is lower than in 2014, where the veal samples were even more resistant to ampicillin (57.9 percent), just like the samples from the white calves (35.6 percent). Furthermore, in some faecal samples from white calves *E. coli* was found with resistance to ceftazidime (0.7 percent) and cefotaxime (2 percent) in 2014, while this was not found in 2015 (Mevius et al., 2015; Veldman et al., 2016).

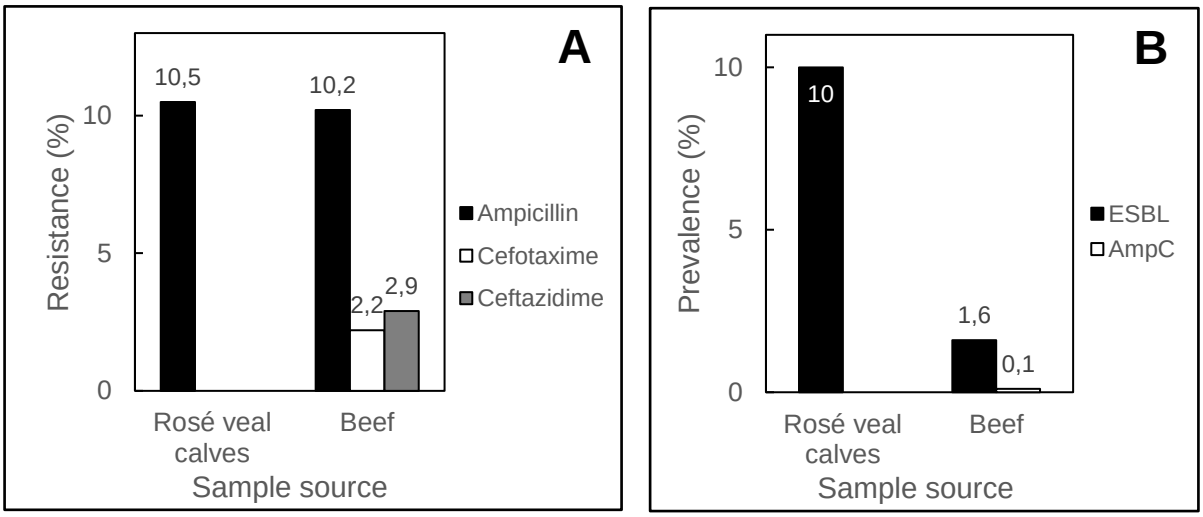

Figure 4.3: The percentage of resistance to ampicillin, cefotaxime and ceftazidime in *E. coli* samples from Dutch rosé calves and beef from Dutch retail in 2015 (**A**) and the prevalence of the resistance mechanisms ESBL and AmpC in *E. coli* samples from these sources in 2015 (**B**).

The prevalence of ESBLs and AmpC in white calves was higher than in the veal samples. Actually, there was no sign of ESBL and AmpC beta-lactamases in the *E. coli* form veal, while – in 2015 – ESBL was confirmed in 16.1 percent of the samples from the white calves and AmpC in 1.2 percent. However, the testing of the *E. coli* from veal was performed with only 21 samples in 2014, which could lead to an underestimation of the real percentage. Therefore, it would be possible that veal could possess ESBL or AmpC producing *E. coli* (Veldman et al., 2016).

The resistance to ampicillin in samples from rosé veal calves (10.5 percent in 2015) is nearly equal to the resistance in the beef samples (10.2 percent in 2015). However, no resistance to both cephalosporins was found in rosé veal calves, while this is present in approximate 2.5 percent of the beef samples on average. The ampicillin resistance in *E. coli* in as well the rosé calves (8.4 percent) as the beef (7.8 percent) was slightly lower in 2014, while the resistance to cefotaxime and ceftazidime are nearly the same as in 2015 (both zero percent in rosé calves; 1.9 and 2.7 percent respectively in beef). Therefore, the ampicillin resistance in rosé calves is approximate the same as in beef, while the difference is made up by resistance to cephalosporins (Mevius et al., 2015; Veldman et al., 2016). (Figure 4.3).

The prevalence of ESBLs in *E. coli* is much higher in rosé veal calves than in beef, in both 2014 (9.1 percent versus 2.2 percent) and 2015 (10 percent versus 1.6 percent). However, in 2015, the AmpC beta-lactamases were present in 0.1 percent of the beef samples, while this did not occur in the rosé calves. Yet, the distinction between ESBLs and AmpC beta-lactamases was not performed with a vast sample size. Therefore, in combination with the very low prevalence of AmpC, no conclusions can be drawn about the AmpC differences between rosé calves and beef. In 2014, much more AmpC was encountered by the researchers in rosé calves (2.2 percent) in comparison to the *E. coli* in beef samples (zero percent). However, the total prevalence of ESBLs and AmpC lactamases in the *E. coli* from rosé calves has nearly not changed from 2014 to 2015, as well as the prevalence in beef (Mevius et al., 2015; Veldman et al., 2016). (Figure 4.3).

Whereas rosé calves were examined in the Netherlands, cattle was examined in Denmark. However, there does not appear to be much difference between the results from the two reports. Ampicillin resistance in the Danish cattle samples is nearly the same as in the *E. coli* in Dutch rosé calves in 2014 (8.4 percent in the Netherlands versus 8 percent in Denmark), and 2.1 percent lower compared to the results of Dutch rosé calves from 2015. In both the Netherlands and Denmark, no resistance to cephalosporins or carbapenems was identified in the samples from the animals. However, whereas ESBLs and AmpC beta-lactamases were found in the *E. coli* from Dutch rosé calves, this was not the case in the Danish samples from cattle (Bager et al., 2015; Mevius et al., 2015; Veldman et al., 2016).

Furthermore, in both countries, the resistances in beef to antibiotics were higher than in the cattle. Similar to the Netherlands, resistance to cephalosporins was also found in the *E. coli* in Danish beef. However, like in the Danish cattle samples, neither ESBL nor AmpC was encountered in the beef samples, whereas this was the case in the Netherlands (Bager et al., 2015; Veldman et al., 2016).

Discussion

In this thesis, there has been strived to determine the influence of antibiotic resistant *E. coli* in the gut microbiome of livestock animals on the food products acquired from these animals. However, it is not possible to formulate one clear answer to this question.

When comparing *E. coli* samples from livestock animals and the corresponding meat to one another, one would expect merely minor differences to appear in the results. Instead, as seen in this thesis, the rates of resistance in *E. coli* differ – sometimes greatly – between the animal and the meat product. Even more – for example in the comparison between rosé veal calves and beef – resistance to cephalosporins occurred in the meat product, while this was not present in the *E. coli* from the calves. Furthermore, the prevalence of ESBLs and AmpC is not always similar – in pigs, this was even around tenfold higher as in pork.

There are several possible causes for these differences. Apart from the – in some cases – low sample sizes – especially in the specifying between AmpC and ESBL – there are some possible explanations for the different amounts of resistance between the animals and the meat. At first, the examined meat samples are not taken from the same animal as the studied faecal samples. This could lead to different rates for the antibiotic resistance and ESBL/AmpC prevalence in the *E. coli* samples between animals and the meat.

In order to avoid this problem, a study should be performed wherein the gut microbiome of several livestock animals should be studied for antibiotic resistance before slaughter by taking faecal samples. After slaughter, samples from the meat of the same animals should be taken and examined in the same manner as the gut microbiome samples. In such a study, the results are not affected by the fact that the meat samples and microbiome samples are not from the same animal. However, no similar study has been performed yet.

Furthermore – as the researchers stated in the MARAN 2015 and MARAN 2016 report – it is possible that samples were taken from imported meat, which also affects the results since the use of antibiotics in the animal sector differs between countries (European Medicines Agency, 2015). However, this issue could also be avoided by performing the possible study mentioned above.

Another issue is the potential contamination in slaughterhouses within and between animal species. Even if only one animal species would be slaughtered, if one individual animal possesses resistant *E. coli* in its gut microbiome, it is possible that all meat could be contaminated with these resistant bacteria. If multiple types of animals are slaughtered in the same place, this could happen between animal species as well. Consequently, the more resistant gut microbiome of chickens could contaminate beef, where normally relatively low amounts of antibiotic resistant *E. coli* are present. This could however be resolved with proper slaughter of the animals in order to keep the contamination as low as possible.

Overall, there are several issues that could possibly affect the outcomes of studies that compare livestock animals with meat products. To obtain completely reliable results, a potential study as mentioned before could be performed. However, the current studies do make clear that the high amounts of antibiotics administered to livestock animals result in the high prevalence of antibiotic resistant bacteria in the gut microbiome, as well as in the meat products

derived from these animals. Since humans consume these meat products, this could have an implication on the human gut microbiome and hence affect the health of humans (Salyers et al., 2007).

Furthermore, it became clear that the resistance levels in Dutch livestock animals and in meat differ from the levels in Denmark, but there are also similarities. The most distinct differences are within the resistance levels of broiler chickens, as well as within chicken meat. The Dutch broiler chickens and chicken meat are contaminated with more than twice as much resistant *E. coli*. For example, the ampicillin resistance in Dutch broilers in 2014 is 62.1 percent, whereas this is 14 percent in Denmark. Also, the ampicillin resistance in Dutch chicken meat is 40.7 percent, while 19 percent of the Danish chicken meat possesses ampicillin resistant *E. coli*. However, the resistance levels in pigs and pork are approximate 50 percent higher in Denmark than in the Netherlands. Furthermore, the resistance levels in Danish cattle are almost equal to the levels in the younger Dutch rosé veal calves. Beef though, possesses more resistant *E. coli* in Denmark than in the Netherlands (for example ampicillin resistance: 11 percent versus 7.8 percent). This all demonstrates that different policies in veterinary antibiotic usage influence the resistance levels in both the animals and the corresponding meat products (Bager et al., 2015; European Medicines Agency, 2015; Mevius et al., 2015).

All in all, more research should be performed to study the effect of antibiotic resistant bacteria in the gut of livestock animals on the food chain and the impact on human health. All these issues start with the high usage of antibiotics in the animal sector. Therefore, changes in the laws and rules around the antibiotic administration could result in a positive effect on the health of the society.

References

Abraham, E. P., & Chain, E. (1988). An enzyme from bacteria able to destroy penicillin. 1940. *Reviews of Infectious Diseases, 10*(4), 677-678.

Alliance to Save our Antibiotics. (2016). *Antibiotic-resistant E. coli on supermarket meat - a serious threat to human health.* Alliance to Save our Antibiotics.

Bager, F., Birk, T., Høg, B. B., Jensen, L. B., Jensen, A. N., Knegt, L., . . . Sönksen, U. W. (2015). *DANMAP 2014 - use of antimicrobiol agents and occurence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark.*

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology, 153*, S347-S357.

Bradford, P. A. (2001). Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews, 14*(4), 933-951.

Butaye, P., Devriese, L. A., & Haesebrouck, F. (2003). Antimicrobial growth promoters used in animal feed: Effects of less well known antibiotics on gram-positive bacteria. *Clinical Microbiology Reviews, 16*(2), 175-188.

CDC Office of Infectious Diseases. (2013). *Antibiotic resistance threats in the united states, 2013.* Centers for Disease Control and Prevention.

Cohen Stuart, J., van den Munckhof, T., Voets, G., Scharringa, J., Fluit, A., & Hall, M. L. (2012). Comparison of ESBL contamination in organic and conventional retail chicken meat. *International Journal of Food Microbiology, 154*(3), 212-214.

Denton, M. (2007). Enterobacteriaceae. *International Journal of Antimicrobial Agents, 29 Suppl 3*, S9-S22.

Drawz, S. M., & Bonomo, R. A. (2010). Three decades of beta-lactamase inhibitors. *Clinical Microbiology Reviews, 23*(1), 160-201.

European Medicines Agency. (2015). *Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013.* European Surveillance of Veterinary Antimicrobial Consumption.

Ewers, C., Bethe, A., Semmler, T., Guenther, S., & Wieler, L. H. (2012). Extended-spectrum beta-lactamase-producing and AmpC-producing escherichia coli from livestock and companion animals, and their putative impact on public health: A global perspective. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases, 18*(7), 646-655.

Fisher, J. F., Meroueh, S. O., & Mobashery, S. (2005). Bacterial resistance to beta-lactam antibiotics: Compelling opportunism, compelling opportunity. *Chemical Reviews, 105*(2), 395-424.

Ghafourian, S., Sadeghifard, N., Soheili, S., & Sekawi, Z. (2015). Extended spectrum beta-lactamases: Definition, classification and epidemiology. *Current Issues in Molecular Biology, 17*, 11-21.

Jacoby, G. A. (2009). AmpC beta-lactamases. *Clinical Microbiology Reviews, 22*(1), 161-82.

Kawamura, K., Goto, K., Nakane, K., & Arakawa, Y. (2014). Molecular epidemiology of extended-spectrum beta-lactamases and Escherichia coli isolated from retail foods including chicken meat in Japan. *Foodborne Pathogens and Disease, 11*(2), 104-110.

Mevius, D. J., Dierikx, C. M., Veldman, K. T., Wit, B., van Pelt, W., Heederik, D., . . . Dorado Garcia, A. (2015). *Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2014 (MARAN 2015).*

Ojer-Usoz, E., Gonzalez, D., Vitas, A. I., Leiva, J., Garcia-Jalon, I., Febles-Casquero, A., & Escolano Mde, L. (2013). Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in meat products sold in Navarra, Spain. *Meat Science, 93*(2), 316-321.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., . . . Kluytmans, J. (2011). Extended-spectrum beta-lactamase genes of Escherichia coli in chicken meat and humans, the Netherlands. *Emerging Infectious Diseases, 17*(7), 1216-1222.

Papp-Wallace, K. M., & Bonomo, R. A. (2016). New beta-lactamase inhibitors in the clinic. *Infectious Disease Clinics of North America, 30*(2), 441-464.

Paterson, D. L. (2006). Resistance in gram-negative bacteria: Enterobacteriaceae. *The American Journal of Medicine, 119*(6, Supplement 1), S20-S28.

Poirel, L., Potron, A., & Nordmann, P. (2012). OXA-48-like carbapenemases: The phantom menace. *The Journal of Antimicrobial Chemotherapy, 67*(7), 1597-1606.

Qin, W., Panunzio, M., & Biondi, S. (2014). Beta-lactam antibiotics renaissance. *Antibiotics (Basel, Switzerland), 3*(2), 193-215.

Queenan, A. M., & Bush, K. (2007). Carbapenemases: The versatile beta-lactamases. *Clinical Microbiology Reviews, 20*(3), 440-58,

Rasheed, M. U., Thajuddin, N., Ahamed, P., Teklemariam, Z., & Jamil, K. (2014). Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. *Revista do Instituto De Medicina Tropical De Sao Paulo, 56*(4), 341-346.

Salyers, A. A., Moon, K., & Schlesinger, D. (2007). The human intestinal tract – a hotbed of resistance gene transfer? part I1. *Clinical Microbiology Newsletter, 29*(3), 17-21.

Vandenbroucke-Grauls, C. M. (2014). Antimicrobial resistance in the netherlands: A natural experiment? *Frontiers in Public Health, 2.*

Veldman, K. T., Mevius, D. J., Wit, B., van Pelt, W., Heederik, D., van Geijlswijk, I. M., . . . Dorado Garcia, A. (2016). *Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2015 (MARAN 2016).*

von Wintersdorff, C. J., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., . . . Wolffs, P. F. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology, 7*, 173.

Walther-Rasmussen, J., & Hoiby, N. (2002). Plasmid-borne AmpC beta-lactamases. *Canadian Journal of Microbiology, 48*(6), 479-493.