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**Measures to reduce ESBL-producing *Escherichia coli* in
pig farms-
a literature review**

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1. Introduction

What role does ESBL-producing *E. coli* play in “One Health” and why does it matter?

Food- and waterborne diseases are causing up to 1.9 million deaths per year in less developed countries. Even in first world countries many cases of illnesses are caused by pathogens which originate from food or water. Most of these pathogens are considered zoonotic (Schlundt et al. 2004) which means that animals can infect humans and vice versa. The way we treat sick animals influences the evolution of the treated germs. In this case we are specifically concerned about the development and transmission of antimicrobial resistance. As a consequence, it is important to keep the One Health aspect of treatment in livestock in mind. *Escherichia coli* (*E. coli*) is a very common bacterial species, which can be nonpathogenic as a normal commensal, in the intestines of humans or animals. Other kinds of *E. coli* like enterohaemorrhagic *E. coli* (EHEC) or Enteropathogenic *E. coli* (EPEC) on the other hand can cause severe infections in animals and humans (Nguyen and Sperandio 2012, Stromberg et al. 2018). In the last years antimicrobial resistance (AMR) in bacteria has increased significantly which limits therapeutic options of diseases caused by these bacteria dramatically (Aarestrup 2015). Extended-spectrum β -lactamases (ESBL) producing *E. coli* are bacteria able to limit the effect of β -lactam antibiotics. By producing enzymes called β -lactamases they inhibit the effect of antibiotics of the penicillin and cephalosporin group (Shaikh et al. 2015). Through mutation of single amino acids extended- spectrum β -lactamases are able to furthermore hydrolyze cephalosporins of the 3rd and 4th generation (Witte and Mielke 2003). Horizontal gene transfer is one of the main mechanisms how antibiotic resistance is spreading.

There are different classifications and genetic variants of extended-spectrum β -lactamases. Not all of the variants are present in *E. coli* in the pig sector. In this research we came across CTX, CMY, OXA, SHV and TEM β -lactamase genes. A classification for β -lactamases, called Ambler-scheme, differentiates between serin- β -lactamases and metallo- β -lactamases. The serin- β -lactamases include ESBL and ampicillinase C (AmpC) β -lactamases. Both are able to inactivate the β -lactam-ring of antibiotics. Resistance against new cephalosporins is often mediated by AmpC β -lactamases (Ambler 1980). A significant difference between ESBL- and AmpC β -lactamases is that β -lactamase inhibitors have little or no effect on AmpC β -lactamases. Carbapenemases on the other hand belong to metallo- β -lactamases (Shaikh et al. 2015). Another way of classification, with more focus on the function of the β -lactamases

was proposed in 1995 by Bush, Jacoby and Medeiros (Bush et al. 1995). They divided the enzymes into four major groups according to their inhibiting abilities. More recently, this classification system was updated to take into account not only substrate and inhibitor profiles but also correlation to the phenotype in clinical isolates (Bush and Jacoby 2010).

The World Health Organization has declared third-, fourth- and fifth-generation cephalosporins as the highest priority critically important antimicrobials for human medicine (World Health Organization 2019). The prevalence of ESBL-producing *E. coli* being resistant to these important cephalosporins has been increasing rapidly over the last decades (Beyer 2014, EFSA 2019). As a consequence, AMR therapy of infections caused by these *E. coli* may become difficult or even fail. Since the prevalence of ESBL-producing *E. coli* is increasing in animals as well as in humans this is becoming a worldwide problem. To address this challenge it is important that veterinarians and doctors of human medicine are working together closely to monitor and limit spreading (Büchter 2011).

There is a lot of evidence on the prevalence of ESBL-producing *E. coli* in livestock, including pigs, and the prevalence in humans. However, the research on measures to specifically reduce ESBL-producing *E. coli* in pigs and prevent transmission is limited.

In this review we aim to

- describe the prevalence of ESBL-*E. coli* in pigs
- describe the current knowledge on factors which contribute to the emergence and spread of ESBL-producing *E. coli* in pigs
- find ways to prevent the emergence and spread of ESBL-producing *E. coli* between
 1. animals on an individual farm
 2. animals and humans
 3. different farms
 4. animals and their environment
- In the discussion we
 1. describe which gaps we found in the literature regarding (biosecurity) measures to limit ESBL-producing *E. coli*
 2. discuss the current knowledge taking into account the quality of these studies.

2. Materials and Methods

To achieve the goal of this review, we developed methods, to find literature on ESBL-producing *E. coli* and to identify relevant studies in this field. Multiple search terms and data bases were used to find the most relevant papers. The databases “Scopus”, “Pubmed” and “Google Scholar” were searched in the time between March 2021 and April 2022. The key words were in English and German and used in the following combinations (swine OR piglet OR pork OR pig OR farrow OR weaner OR sow OR Schwein OR Sau OR Ferkel OR Eber OR Mäster OR Läufer) AND (*E. coli* OR ESBL OR Extended-Spectrum-Betalactamases OR *Escherichia coli* OR Erweiterte-Spektrum Lactamasen) AND livestock AND (measures OR intervention OR biosecurity OR Maßnahmen OR Biosicherheit). Initially, the results were screened by the title to see if the study matches the criteria and might be relevant for the topic. There were no limitations on the study design. The abstracts of the papers that met our requirements were then read to see if the findings would contribute to our review. As the main criterion we looked specifically for papers on ESBL-producing *E. coli* and not just any *E. coli* in pig farms. We tried to find data on the topics prevalence, risk factors, ways of spreading between animals, on farms and onto other farms. Beside that we also wanted to cover the relevance for humans and factors of ESBL-producing *E. coli*. The search was limited to pigs. Data on other animal species or bacteria other than *E. coli* / Enterobacteriaceae were excluded from this review. In some cases, the references of the papers were also looked into if they seemed relevant for our research. After this procedure the identified literature was collected, and the citations were exported in the bibliographic database “Citavi”.

In most of the papers included in this review the main focus was on prevalence and based on these findings hypotheses on transmission routes were made. Because there is little evidence on this specific topic, we also took some studies regarding other forms of antimicrobial resistance into account. Since ESBL-producing *E. coli* belong to the group of Enterobacteriaceae, research on resistant Enterobacteriaceae was also included and if possible, just the specific results for *E. coli* were considered. In some cases, we assume that the behavior (e.g. transmission through air) of resistant bacteria other than ESBL-producing *E. coli* would be the same. We included some of these results on wider topics but always suggested that further research on this and whether this behavior also applies to ESBL-producing *E. coli* is necessary.

Due to the many different terms and categories used for ESBL-producing *E. coli* we included research with different expressions and specialties and tried to maintain the precision. Some studies for instance did not differentiate between ESBL and AmpC producing *E. coli* and just used the term “ESBL/AmpC producing *E. coli*”. In cases like this, we used the exact terms of the paper, so no inaccuracy occurs. Terms that have no official definition came up and are sometimes used interchangeably/loosely which made it difficult to ensure precise language. One of these terms is “multidrug resistance (MDR)”, which is defined as resistance to three or more antimicrobial classes by the European Food Safety Authority (EFSA) (EFSA 2019) but is also sometimes used as term for resistance to three or more antimicrobials. By ensuring to use the same terms as in the used literature we tried to prevent misunderstandings.

3. Results

3.1. Prevalence

Overall, numerous studies were published in recent years regarding the prevalence of ESBL-producing *E. coli*, which followed different layouts, applied different methods and ways to present the outcomes. There are differences in the outcomes of the studies which may reflect to some extent differences in the specific study population, region, sampling methods and analytics. The prevalence in pigs differs from the prevalence in humans but the differences between studies is bigger. Some of them are described below and presented in **Table 1** (pigs) and **Table 2** (humans).

3.1.1. Prevalence in pigs

One study took place at the German-Dutch border and sampled 35 pig farms. Overall, the study team took 540 rectal swabs to detect ESBL-producing Enterobacteriaceae. The samples were taken from two different compartments on the farm, housing different age groups. On 22 farms, 10 samples (one swab per pig) in each compartment, were collected while on the other 13 farms only 5 samples per compartment were collected. It showed that ESBL-producing Enterobacteriaceae were detectable in 30.2 % (163/540) of the pigs. The study team characterized the ESBL-producing strains further and found that the majority with 95.1 % were *Escherichia coli* isolates. They also detected a few other species like *Citrobacter*, *Serratia fonticola* and *Enterobacter cloacae*. Overall, 74.3 % (26/35) of the farms investigated had pigs being colonized with ESBL-producing Enterobacteriaceae while only 25.7 % (9/35) did not have any ESBL-producing Enterobacteriaceae being retrieved of their samples (Schmithausen et al. 2015b).

In a German screening study, 7 out of 16 (43.8 %) fattening farms in north-eastern Germany were positive for ESBL/AmpC producing *E.coli* (Friese et al. 2013). Just these 7 farms that tested positive for ESBL/AmpC producing *E. coli* were included in the following study. As a result, they had a prevalence of 37 % ESBL/AmpC positive samples. Results were based on a method including enrichment of the 420 individual faecal samples they took (Salviati et al. 2014).

Hering and coworkers sampled fattening pigs on 48 commercial farms in different parts of Germany. Three faecal samples were taken from the floor of two groups at each farm respectively and then pooled. The samples were categorized as ESBL suspicious if colonies grew on agar containing cefotaxime and were furthermore confirmed as *E. coli* by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) and subsequent confirmation of ESBL genes. The prevalence of positive farms was 85 % (41/48), while the prevalence of the faecal samples was 61 % (177/288) (Hering et al. 2014).

A German study conducted in 2005 and 2006 used a different sampling approach. Cooperating with the BVL (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit) they had 652 different *E. coli* strains from diseased pigs from an AMR monitoring program available. Samples of animals that got up to 4 weeks prior to sampling antimicrobial treatment were excluded from the study. Pig samples with the following indications were included: respiratory diseases, enteritis, urogenital infections, septicemias, and other diseases. The primary samples from diseased animals had been cultivated on non-selective agar to detect *E. coli* and provided for further analyses. In a second step, for each *E. coli* isolate a microdilution procedure was used to determine the Minimum Inhibitory Concentration (MIC) of the different antibiotics. ESBL confirmation was performed by disc diffusion according CLSI standard. Applying PCR they were able to confirm 1.2 % (11/652) strains harboring ESBL genes (Büchter 2011).

Since the prevalence of resistant *E. coli* has a high variation between regions, we also included studies from other countries. Beside methodological differences, these differences may reflect different hygiene standards and handling of antibiotics. A study in northern Vietnam found 89 (89 %) of the 100 sampled farms had cefotaxime-resistant *E. coli* (CREC) in the faecal samples of their pigs. The samples of each farm (five pigs from each of 3 pens) were pooled and after selective plating about 5 colonies were picked and biochemically confirmed as *E. coli*. Resistance to cephalosporins was identified through disc diffusion. Two resistant isolates were confirmed as ESBL-suspicious by modified double-disc synergy test, and finally subjected to PCR for ESBL gen confirmation. In this area, β -lactams including third- and fourth-generation cephalosporins are commonly used in humans and livestock. Growth promoters were used regularly in these regions and it seemed that the farmers instead of the veterinarians often made decisions on the drugs used. In the questionnaire only 73 % of the farmers declared that they often ask the veterinarians for advice in case of sick pigs. Also, the dosages were more frequently decided by the farmers than the veterinarians. The farmers declared that the

dosages reached from 0.5 up to double the amount of the recommendation of the drugs (Dang et al. 2018).

A Korean study obtained samples from 19 pig farms, slaughterhouses, workers, and environment in 2017 and 2018. The overall prevalence of ESBL-producing *E. coli* (including human samples and meat samples) using selective plates was 14 % (232/1614). The most common ESBL type was bla_{CTX-M-55}. The samples were retrieved from different regions in Korea. In total the prevalence of ESBL-producing *E. coli* in pig samples (nose, skin (groin region), rectum, and stool of swine) was 18.4 % (140/760). The environmental samples were collected from fences of the stable, fan, floor and soil around the stable. The prevalence of the environmental samples of all regions was 20.6 % (28/136). However, the results were not broken down on which sample results were from which spot of the environment (Kim et al. 2021).

In a study conducted in Great Britain 56 pig farms were sampled in 2014 to 2015. The *E. coli* retrieved from selective and non-selective plates were then tested for genes that mediate antimicrobial resistance. Of the total of 492 *E. coli* collected from samples of farrow-to-finish farms and finisher farms only 28 *E. coli* isolates had genes mediating resistance to extended-spectrum cephalosporins (ESC). Some *E. coli* harbored two extended-spectrum cephalosporin genes. Additionally, 16 *E. coli* were found that had mutations in the AmpC promotor region which also promotes resistance to β -lactam-antibiotics by expressing β -lactamases. It should be considered that the samples were only taken from healthy slaughtered pigs which might show a distorted picture of the resistance situation. In conclusion 9 (16 %) out of 56 farms had ESC resistance genes in the sampled *E. coli*. Out of 492 samples 28 (5.7 %) from 9 different farms had ESC genes and 16 (3.3 %) mutations in the AmpC region. ESC genes detected were bla_{CMY-2}, bla_{CTX-M-1}, bla_{CTX-M-15}, bla_{SHV-12} (AbuOun et al. 2020).

The prevalence in Denmark was thoroughly researched by Hammerum and coworkers. Samples were retrieved from 20 farms with no third- or fourth-generation cephalosporin use and from 19 farms that had used these antibiotics recently. 15 faecal samples were collected from different age groups on each farm. The samples were pooled into groups of 5. Divided into the two sample groups, the second group had a significantly higher prevalence of ESBL-producing *E. coli* being detected on the farm than the group that did not use any third- and fourth-generation cephalosporins recently with 79 % (15/19) and 20 % (4/20), respectively (Hammerum et al. 2014).

In a cross-sectional study in the Netherlands 40 multiplier farms (27 of them with finishers) were investigated using selective culturing. The production types were farrow or farrow-to-finish farms. They sampled closed and open farms (getting gilts from suppliers) of both types. To determine the prevalence the researchers took rectal swabs of 60 pigs of different age classes at each farm. For the analyses 6 samples of the same stable were pooled resulting in ten samples per farm. Out of the 40 farms, from 23 farms phenotypic cephalosporin-resistant *E. coli* were isolated, and from 18 farms presence of ESBL genes was confirmed. ESBL gene presence in pooled samples ranged from 16% in (rearing) gilts to 29% in sows, suckling piglets and finishing pigs (Dohmen et al. 2015).

In a consecutive longitudinal study in the Netherlands 36 conventional farms with different farm types were included. The study consisted of four sampling events in a period of 18 month. The results showed an overall prevalence of 27 % (pools positive for ESBL-producing *E. coli*) in pigs at the first sampling moment and a significantly lower prevalence of 13 % of pools at the last sampling date (Dohmen et al. 2017a). Graphical display of the number of positive samples at each sampling moment shows that for each of the farm types a decrease could be observed. Prevalence in pooled samples was highest for farrow-to-finish open farms compared to other farm types. The assessment of the farms as a whole also showed a significant decrease in farms being classified as ESBL-producing *E. coli* positive between 2011 and 2013. While at the first sampling moment 44 % (16/36) of farms had pigs carrying ESBL-producing *E. coli* at the last sampling moment they found only 28 % (10/36) of farms to be positive. Interestingly 19 farms stayed negative throughout the whole study, 7 farms became negative and one farm became positive for ESBL-producing *E. coli* (Dohmen et al. 2017a).

Table 1: Prevalence of ESBL-producing *E. coli* in pigs from different studies

Country	Year	Prevalence	Samples	Additional info	Reference
German-Dutch border	2012	74.3 % (26/35) ^a ESBL-producing Enterobacteriaceae 30.2 % (163/540) ^b ESBL-producing Enterobacteriaceae	Rectal swabs	95.1 % of the Enterobacteriaceae were <i>E. coli</i> SEL	(Schmithausen et al. 2015b)
Germany	2012	37 % (155/420) ^b ESBL/AmpC producing <i>E. coli</i>	Faecal samples (with enrichment)	Prior testing- just the farms with ESBL-producing <i>E. coli</i> were assessed SEL	(Salviati et al. 2014)
Germany	2011-2012	85 % (41/48) ^a ESBL suspicious <i>E. coli</i> 61.5 % (177/288) ^b ESBL suspicious <i>E. coli</i>	Faecal samples, boot swabs, dust samples Faecal floor samples	SEL SEL	(Hering et al. 2014)
Germany	2005-2006	1.2 % (11/652) ^c ESBL-producing <i>E. coli</i> (among all <i>E. coli</i>)	Different samples including tissue and faeces	Study on available isolates from diseased animals NON SEL	(Büchter 2011)
Korea	2017-2018	18.4 % (140/760) ^b ESBL-producing <i>E. coli</i>	Nose, skin, rectum, and stool	SEL	(Kim et al. 2021)
Vietnam	2015	89 % (89/100) ^b Cefotaxime-resistant <i>E. coli</i>	Rectal swabs, mixed samples of each farm	Samples of each farm mixed (5 rectal swabs together) SEL	(Dang et al. 2018)
Great Britain	2014-2015	5.7 % (28/492) ^c ESC	Caecal content, 10 samples of	Samples from healthy slaughter pigs SEL and NON SEL	(AbuOun et al. 2020)

		3.3 % (16/492 <i>E. coli</i>)^c <i>E. coli</i> with mutations in the AmpC region	each herd pooled		
Denmark	2010-2011	<u>No cephalosporins used:</u> 7.3 % (13/177)^b ESBL-producing <i>E. coli</i> <u>High consumption of cephalosporins: 30.9 % (50/162)^b ESBL-producing <i>E. coli</i></u>	Faecal samples, 5 samples pooled each	SEL	(Hammerum et al. 2014)
Netherlands	2011-2013	27 % (2011) -13 % (2013) (sample sizes missing)	Rectal swabs, 6 samples pooled each	Study was conducted 2011-2013 and a considerable decrease was noted SEL	(Dohmen et al. 2017a)

Sampling moment is noted under "Year".

SEL= If selective methods were used; NON SEL=if nonselective methods were used for growing *E. coli*.

^a= prevalence refers to number of farms; ^b= prevalence refers to number of samples; ^c= prevalence refers to number of *E. coli* isolates

The prevalence of ESBL-producing *E. coli* in pigs seems to be different from the prevalence of ESBL-producing *E. coli* in meat. Although there is evidence that the two prevalences correlate, ESBL-producing *E. coli* occur less frequently in meat than in pigs. Bergšpica and coworkers calculated a high correlation level between *E. coli* suspected to be ESBL-producing in pigs and *E. coli* suspected to be ESBL-producing in pork meat from data of 2017 and 2015. The differences in the detected prevalence are most likely because ESBL-producing *E. coli* occur in the intestines and the harvesting process (slaughter) for meat includes several hygiene steps, including removing the intestines (where cross contamination is avoided). Furthermore, most samples to identify resistant *E. coli* are taken from rectal swabs or intestinal contents where the most *E. coli* are found instead of skin swabs for example (Bergšpica et al. 2020). The data on the prevalence of ESBL-producing *E. coli* in pork meat varies a lot in different countries in the European Union (EU) as well as in different publications. For example the prevalence in 2015-2016 in meat from one slaughterhouse in Belgium varied between 25 % and 1 % when different parts of the carcasses were sampled (Biasino et al. 2018).

3.1.2. Prevalence in humans with a special focus on people working in the pig sector

The transmission of ESBL-producing *E. coli* from pigs to humans is not completely clear. There are several findings that transmission of ESBL-producing *E. coli* from pigs to humans is likely. Similar to pigs there might be different transmission paths including direct contact, air/dust and meat as food. In a Dutch study they identified direct contact between humans and animals as the main way of transmission. In the collected dust samples, they detected the same ESBL genes as in the ESBL-positive pigs and as in the ESBL-positive people. Therefore, one possibility is that dust/air is a way of transmission. On the other hand, most of the participants which tested positive for these ESBL genes also had direct contact with the pigs. This makes it difficult to identify if the way of transmission was by air/dust or by direct contact and faecal-oral intake. When the informative value of this study is discussed it should be considered that just 3.6 % of the people tested positive, making these results probably hard to replicate (Dohmen et al. 2017b).

Moodley and Guardabassi found the resistance gene CTX-M-1 in pigs and in 75 % (3/4) of humans having contact with them, pointing to transmission (Moodley and Guardabassi 2009).

The prevalence of ESBL-producing *E. coli* has a high variability in different studies and countries. The overall prevalence in the country and the sampled population seems to have a major impact on the results.

In Estonia a study on 207 (138 in contact with animals, 69 without contact) individuals showed 14 (6.8 %) stool samples being colonized with ESBL-producing *E. coli*. Interestingly, seven out of 29 sampled pig farmers (24.1 %) were colonized, which led to the assumption/conclusion that pig farmers have a significantly higher prevalence than the average studied population. The sampled population included veterinarians, dog owners and pig farmers. The control group which had no contact to animals consisted of 69 participants showed a prevalence of 5.8 % (4/69). The most common ESBL gene in this study was bla_{CTX-M-1}, being isolated primarily from pig farmers (Telling et al. 2020).

The already mentioned Danish study from Hammerum and coworkers sampled 195 people from 19 different farms to investigate the prevalence of ESBL-producing *E. coli* in humans. 19 of the participants showed ESBL-producing *E. coli* in their faeces. Interestingly, all of the positive samples except one were from humans that had contact with pigs. The majority (14/19) of the people being colonized with ESBL-producing *E. coli* worked at farms that used a high amount of third- and fourth-generation cephalosporins. Additionally, there was a correlation of

the prevalence between people who had direct contact with pigs being colonized with ESBL-producing *E. coli* versus people that worked with pigs where no ESBL-producing *E. coli* were found. In the first group 21 % (15/72) had ESBL-producing *E. coli* in their stools, while the group where the pigs were supposedly not colonized showed just 5 % (3/64) of the people had ESBL-producing *E. coli* in their stools. In contrast on three farms (3/39) the humans tested positive for ESBL-producing *E. coli* while the pigs did not (Hammerum et al. 2014).

The Vietnamese study of Dang and coworkers showed a high prevalence of CREC in pigs. Regarding the farm owners 86 (86 %) of the human faecal samples contained cefotaxime-resistant *E. coli*. As mentioned above these high results may be due to an intensive use of cephalosporins, especially if administered by non-medical staff (Dang et al. 2018).

A study conducted in different regions of Korea showed an overall prevalence of 9 % (16/178) in workers of the pig industry. The prevalence in the different regions in Korea varied between 0 % (0/44) and 18.8 % (3/16). Swab samples from workers were collected from nose and various skin areas. Additionally, stool samples from 45 workers and pooled stool samples from a toilet were obtained (Kim et al. 2021).

Dohmen and coworkers did a previously described study on the prevalence and risk factors of ESBL-producing *E. coli* on 40 pig farms. Furthermore, the study team did a second study on the same farms to find out the carriage of ESBL-producing *E. coli* in people working and living on these farms. The samples were collected from 142 people with faecal cups. The results showed a prevalence of 6 % (8/142). 8 humans carried ESBL genes which were detected in 12 isolates. The samples showed identical or similar ESBL genes in the pigs and humans pointing once more to the possibility of transmission (Dohmen et al. 2015).

Sampling people at 31 pig farms in Switzerland, Moor and coworkers found a prevalence of 6.8 % (5/73) in the human samples. At their farm visits they asked the farmers and farm workers to send in stool samples. On average 1.3 stool samples per farm were sent in, adding up to 73 samples in total. Comparing the result of 6.8 % prevalence to the results of a Swiss prevalence study on ESBL-producing *E. coli* in healthy humans with 5.8 % (Geser et al. 2012) they concluded that there was no elevated risk of being ESBL positive when working at a farm (Moor et al. 2021). Interestingly, some human samples had extended-spectrum cephalosporin-resistant *Escherichia coli* (ESC-R-EC) positive samples although in their associated farm the pig samples were negative. There was no difference in prevalence of the human samples whether they were working in maternity sections or fattening section (Moor et al. 2021).

Table 2: Prevalence of ESBL-producing *E. coli* in humans from different studies

Country	Year	Prevalence	Samples	Additional info	Study
Vietnam	2015	86 % (86/100) cefotaxime resistant <i>E. coli</i>	Individual stool sample	SEL	(Dang et al. 2018)
Korea	2017-2018	9 % (16/178) ESBL-producing <i>E. coli</i>	Pooled stool sample in toilet, skin swabs	SEL	(Kim et al. 2021)
Denmark	2010-2011	10.3 % (19/195) ESBL-producing <i>E. coli</i>	Rectal swab	SEL	(Hammerum et al. 2014)
Netherlands	2011	6 % (8/142) ESBL-producing <i>E. coli</i>	Faecal samples	SEL	(Dohmen et al. 2015)
Switzerland	2018-2019	6.8 % (5/73) extended-spectrum cephalosporin-resistant <i>E. coli</i>	Individual stool samples	SEL	(Moor et al. 2021)

Sampling moment is noted under "Year".

SEL= If selective methods were used; NON SEL=if nonselective methods were used for growing *E. coli*.

All the prevalences refer to number of samples as a unity

3.2. Factors influencing the introduction of ESBL-producing *E. coli* into farms

There are many ways where pigs may come in contact with new ESBL-producing *E. coli*. In the first step of transmission the pathogen is introduced to the farm. From this point on the pathogen may be able to further spread on the farm e.g. between compartments.

3.2.1. Transmission routes between farms

People coming to the farm are a transmission route that needs to be considered. Whether they are workers or visitors they can introduce new pathogens to a farm. Hygiene locks are one way to prevent this from happening. A hygiene lock as the only entrance to the farm showed

a significant decrease in the prevalence of ESBL-producing *E. coli* in a Dutch study (Dohmen et al. 2017a). Visitors could pose a risk for external biosecurity by transmitting resistant bacteria that they might harbor themselves, have acquired from other animals or might even have been picking up on their way (for example with their vehicles).

Gay and coworkers did a study on the effects of different hygiene measures on the occurrence of ESBL-producing Enterobacteriaceae (the majority in this study being *E. coli*). They looked at different measures and found out that some increase the occurrence of ESBL-producing Enterobacteriaceae and therefore can be considered as risk factors while others decrease the occurrence of ESBL-producing *E. coli* seeming to have a protective effect. The measures that increased the occurrence of ESBL-producing Enterobacteriaceae were “farmers visits”, “soak the floor” and “recent antibiotic use (<1 year)” (Gay et al. 2018).

Visits of farmers compared to the general population might be an even bigger risk because they have more intensive contact with animals and other people working with animals such as veterinarians and staff of animal transporters. They can act as a transmission pathway for resistant bacteria. Gay and coworkers also put forward the theory that other farmers visiting the farm are not the main issue but shows more so a higher frequency of overall visits which could also be the critical factor that increases the occurrence of ESBL-producing Enterobacteriaceae (Gay et al. 2018).

When new pigs are bought the whole process holds risk for transmission of pathogens. Trading places where lots of different animals and their microflora come together often lead to colonization with new pathogens. Livestock transport vehicles can be a route of transmission if they are not correctly cleaned and disinfected. They may lead not just to distribution in, but also between regions (Bergšpica et al. 2020, Schmithausen et al. 2018). The probability of the transmission of ESBL-producing *E. coli* during transport and the waiting period at the abattoir lies nearly at 30 %, although the waiting situation may influence the probability of transmission (Schmithausen et al. 2015b). It needs to be considered that pigs usually undergo several production steps and are transported to different areas where they might acquire different germ spectrums (Schmithausen et al. 2018).

Finally, the introduction of new animals into herds plays a big role in transmission. They bring their own germ spectrum from their old stable to the new herd (Bergšpica et al. 2020). Therefore, they also bring their own antibiotic-resistant bacteria which may be resistant to other antimicrobials than what the new herd has been in contact with.

3.2.2. Farm factors and production regions

There are many factors that influence the occurrence of ESBL. The influence of farm-related factors is not completely clear. One study showed that regarding the farm environment presence of waterfowl, pig farms in the surrounding area, farm administration, hygiene, size of farm, size of sampled animal group, feed, persons attending animals and of course the use of β -lactam antibiotics had a significant correlation with the occurrence of ESBL (Hille et al. 2018).

An Indian study investigated the effects of the organization level of farms on the occurrence of ESBL-producing *E. coli*. One group of 40 unorganized pig farms was compared to 58 organized farms in Mizoram, India. Using the double-disk synergy test method, 170 of the 258 isolated *E. coli* were found to produce ESBL. In total out of the 258 isolated *E. coli* in 29 isolates at least one ESBL gene was found. Comparing the two farm systems based on results with the screening test method, 82/120 (68.33 %) positive samples were taken from the organized farm while the unorganized farm had 88/138 (63.77 %) samples positive for ESBL-producers. Additionally, the resistance against third-generation cephalosporins was reported to be significantly higher in unorganized farms which is not reflected in the data presented. As regards overall resistance rates, higher levels were observed in unorganized farms. The explanation for this might just be that these antibiotic classes are more frequently used in unorganized farms (Mandakini et al. 2020). Regarding the informational value it should be considered that there was no significant difference between the prevalence of ESBL producers in the two groups with 68.3 % and 63.7 %, respectively. Although the increased resistance against several antimicrobials in unorganized farms points in a direction this information should not be overrated. The paper does not provide any definition of what “unorganized and organized farms” are specifically.

There appears to be a correlation of the location of a farm and the occurrence of ESBL-producing *E. coli*. The impact of the production region on resistance seems to be due to the density of pig production in the surroundings area. For example, the farms in the Southeast of Great Britain had a significantly lower risk of multidrug resistant *E. coli* than the ones in Yorkshire and the Humber region, whereas the Southwest regions were less likely to have MDR isolates in their samples. These findings correlate with the density of production as the Southwest is just sparsely populated, while Yorkshire and Humber have a lot of farms (AbuOun et al. 2020). A German study on cefotaxime-resistant *E. coli* had similar results. The factor “a

forest within a radius of 1 km” showed a positive effect on the infection rates. A forest in the surrounding might indicate less production density and less other farms nearby which would explain the effect due to lower infection pressure (Hille et al. 2017).

A study in Great Britain on 56 pig farms in 2014 to 2015, described already earlier, tested antimicrobial resistance in *E. coli*. Beside sampling the pigs, the farmers were asked to fill out a questionnaire on farming practices. The statistic showed that 6 of the 29 potential risk factors were significantly associated with the occurrence of MDR *E. coli*. Although their study was on resistance in *E. coli* and not specifically on ESBL-producing *E. coli* it is reasonable to assume that the risk factors may also apply to ESBL-producing *E. coli*. Interestingly, in this study specialist finisher farms had a higher probability of testing positive for MDR *E. coli* than breeder-finisher farms. One explanation for this phenomenon could be the management of the farms. Finisher farms may have a higher number of contacts with other farm-related people and vehicles which may be the cause of more infections with MDR *E. coli* (AbuOun et al. 2020). The fact that finisher farms have a higher prevalence of MDR *E. coli* might also be explained by the different origins of the pigs mixed in the compartments. This system might act as mixing pot for pathogens from the different farms the pigs are supplied from.

3.2.3. Slurry used as fertilizer and biogas production

The common practice of slurry being used as fertilizer for fields may pose another risk of transmission of resistant *E. coli* into the environment and back to farms. Several studies were conducted to find out if ESBL-producing *E. coli* are common in faeces, slurry and subsequently on fertilized fields. Friese and coworkers did research on German pig farms. They sampled 16 pig fattening farms and 16 pig breeding farms. After pooling the samples, the pig breeding farms had a little higher rate with 56.3 % of positive samples for ESBL/AmpC producing *E. coli* than the pig fattening farms with 43.8 %. Due to the small samples size in the observed prevalence no statistically significant difference between the different production types of farms could be confirmed. As the next step they sampled the slurry at the fattening pig farms and were able to detect ESBL/AmpC-producing *E. coli* in all (100 %) of the five tested slurry samples. Furthermore, they sampled the surfaces of fields that were fertilized with slurry within the last six weeks. As sampling method boot swabs were used. They were able to detect three phenotypically suspicious ESBL/AmpC-producing *E. coli* carrying bla_{TEM} and bla_{SHV} genes. The positive samples were retrieved from three different spots on a fertilized field in the area of one

pig farm (Friese et al. 2013). This research shows impressively how ESBL/AmpC-producing *E. coli* can be distributed into areas surrounding pig farms. This illustrates that not only the transmission onto humans but also onto pig farms in the same area might occur.

Salviati and coworkers had similar results when sampling slurry on partly the same German pig fattening farms. The slurry samples showed a high prevalence of being contaminated with ESBL/AmpC producing *E. coli*. Overall, 14/17 (82.4 %) of the slurry samples tested positive for ESBL/AmpC producing *E. coli*. Slurry is usually used as fertilizer which therefore poses a risk of contamination of the environment. Furthermore, four samples of digestate from biogas plants were tested. Out of these, 3/4 (75 %) of them tested positive for ESBL/AmpC producing *E. coli*. It should be considered that the four samples were just from two different farms and that all the positive samples were retrieved from the same farm. The form of treatment for the biogas plants was mesophilic digestion which appears to not be eliminating the tested bacteria (Salviati et al. 2015).

Furthermore, in the same study, 87 boot swabs in the vicinity of the farms were taken. The samples were taken from various ground with different distances from the stable including fields (some have been fertilized with slurry), meadows, roads, and grass strips. As a result, 14/87 (16.1 %) of the boot swabs had ESBL/AmpC producing *E. coli* on them. The majority of the positive samples was from the sampling point with the smallest distance (50m), but two samples with ESBL/AmpC producing *E. coli* were even found at a distance of 500m on the downwind-side. The sampled areas also included fields of which some have been fertilized with slurry. Interestingly all of the fields that tested positive for ESBL/AmpC *E. coli* have been fertilized about one month prior to sampling, suggesting that ESBL/AmpC *E. coli* survive over a long period of time. These findings suggest that fertilizer might play a role in transmission of ESBL/AmpC producing *E. coli* in the surroundings of farms with distances up to 500m. Since ESBL/AmpC producing *E. coli* have also been detected from areas that have not been fertilized Salviati and coworkers suggested that moving animals and vehicles might be responsible for the contamination of these surfaces (Salviati et al. 2015). As additional investigation on the relatedness of the detected ESBL/AmpC producing *E. coli* Pulsed-field gel electrophoresis (PFGE) was applied and showed that the isolates from the fields (boot swabs on fields) and the slurry contained the same genes and PFGE-profiles. This suggests that all detected ESBL/AmpC producing *E. coli* indeed are emitted from the sampled pig farm (Salviati et al. 2015).

The results of a study conducted in Switzerland is in line with the hypothesis that manure is a potential way of transmission of resistant bacteria into the environment. In this study Moor and colleagues looked for ESC-R-EC. They detected ESC-R-EC in 16.7 % of the liquid manure samples (n=10) (Moor et al. 2021).

When surface of the soil where pigs roam was swabbed ESBL-producing *E. coli* have been found (Samanta et al. 2018). The environment probably gets contaminated through faeces. This risk of soil and water being contaminated with ESBL-producing *E. coli* should be considered if outdoor enclosures are used.

3.2.4. Water

Drinking water, waste water and surface water can all be contaminated with ESBL-producing *E. coli*, and can be another source of transmission (Bergšpica et al. 2020).

It seems likely that drinking water for pigs plays a role in the transmission of ESBL-producing *E. coli*. The study of Samanta and coworkers reinforced this hypothesis by showing that the drinking water for pigs in India was contaminated with ESBL-producing *E. coli* (Samanta et al. 2018). Its quality needs to be assessed carefully since Dohmen and coworkers found out that water from public sources had a positive effect on the likelihood of ESBL-producing *E. coli* while a supply from private sources increased the prevalence (Dohmen et al. 2017a).

A Canadian study researched the impacts of different factors including livestock on private drinking water sources in terms of the occurrence of resistant *E. coli*. Antibiotic resistance was tested on a general level but there were no tests to specify the resistance mechanisms. The factor “pigs on the property” was significant (p-value <0.03) for occurrence of resistance to 1 or 2 classes compared to fully susceptible *E. coli*. Regarding multiclass resistance (3 or more classes) the factor “pigs on property” was even more significant with a p-value of <0.001 (Coleman et al. 2013).

3.2.5. Air/Wind

Several diseases (e.g. foot-and-mouth disease) are known to be able to spread through air/wind (Cannon and Garner 1999). To evaluate the risk of ESBL-producing *E. coli* being distributed through ambient air onto farm environment Salvati and coworkers sampled air in

and outside of a pig stable. The outdoor samples on the upwind (distance of 100 m) and the downwind side (distance of 50 m) were taken at each farm over the period of 90 minutes at each sampling moment using impingement. Out of 36 samples they detected ESBL/AmpC producing *E. coli* in one sample from the upwind and one sample from the downwind side of them (6 %, 2/36). Furthermore, they took boot swabs of the ground surrounding the farm at different distances from 50m to 500m to the stable. As mentioned above the swabs included fertilized fields but also unfertilized grounds. The analysis of the boot swabs showed that there were ESBL/AmpC-producing *E. coli* present in 26.1 % (14/87) of the samples. Samples from the downwind side showed to have positive results at 50 m, 150 m, 300 m and 500 m. The upwind side was sampled only at the 100 m mark, nonetheless, there were also ESBL/AmpC producing *E. coli* detectable (Salviati et al. 2015). The detection of ESBL/AmpC producing *E. coli* in the air samples outside the stable shows the possibility of transmission and spread through air. If other farms are close by there might be a chance of introduction of the *E. coli* through ventilation. It is unclear if the resistant bacteria found on boot swabs of the ground were particles that sedimented to the floor after being emitted by exhaust air or if the transmission was through direct contact, for example by a wheelbarrow. Detecting ESBL/AmpC producing *E. coli* in the samples from the ground at a distance up to 500 m indicates a chance of transmission through boots or vehicles touching the ground and carrying resistant bacteria to the next farm. This risk should be considered when vehicles, visitors or workers enter the farm.

3.2.6. Wildlife, pets and other livestock

Wild animals can function as vectors of many diseases (or their agents) as well as introduce AMR resistant bacteria. Studies have shown that different wild animals can harbor resistant *E. coli* and be a threat for the farms. *E. coli* with reduced susceptibility to different antimicrobials including ampicillin (β -lactam antibiotic) were found in wild bird faeces surrounding a pig farm (Lucia et al. 2021). A study on birds in Germany revealed the presence of ESBL-producing *E. coli* in 2.3 % of the sampled animals. Four birds of different species, out of the 172 wild birds sampled, tested positive for ESBL-producing *E. coli*. The detected β -lactamase genes were bla_{CTX-M-15} and bla_{TEM-1} which are also commonly found in ESBL-producing *E. coli* in pigs (Guenther et al. 2010). The results show that wild birds contribute to the distribution of ESBL-producing *E. coli* and there might be transmission to livestock or vice versa.

In areas where racoons are resident, they may pose a risk for pig farms since they have been shown to carry ESBL-producing *E. coli* on their paws and distribute it through their faeces. Interestingly, in a study carried out in Southern Ontario more ESBL-positive racoon samples were from conservation areas than from swine farms. Also, the prevalence of resistant *E. coli* detected on the paws of the racoons sampled in the conservation area were unexpectedly higher than the ones sampled on the swine farms. This may be caused by their large home ranges which led to racoons being captured that do not frequently roam around this farm. Because of their large home ranges, the risk of ESBL-producing *E. coli* being spread across long distances seems possible. Some ESBL genes (bla_{CTX-M} and bla_{CMY-2}) were exclusively found on the conservation areas (soil and racoon samples) and not on the farm areas which led the authors to suggest that these genes came from a human source. The samples of the pig farms were retrieved from soil and the manure pit of each farm however, the pigs on the farms were not sampled. The only ESBL gene that was found in the environment of the pig farms (and not just in the faeces or paws of racoons) was bla_{TEM} . It was found in the soil samples, the sample of the manure pit and in the racoon faeces, suggesting that this resistance gene is present on the pig farm. However, the overall prevalence of resistant *E. coli* found in faecal samples of racoons were equal in conservation areas and pig farms (Bondo et al. 2016).

A systematic review analyzed published studies on the contacts of wildlife mammals with livestock. Looking at the data of different sources they identified interactions between livestock pig and the following mammals: deer, canines, wild pigs. In the literature they found indirect as well as direct contacts between all the mentioned wildlife and pigs (Bacigalupo et al. 2020). These wildlife animals need to be considered as potential vectors for ESBL-producing *E. coli*, especially when direct contact with livestock might occur.

Pets also pose a risk of acting as carriers of ESBL-producing *E. coli*. Although the sample size is very small, Salviati and coworkers were able to detect ESBL/AmpC producing *E. coli* in 2/2 (100%) of the sampled dog faeces on two different farms. The faeces showed the same resistance gene (CTX-M-1) as the majority of the ESBL/AmpC positive samples of the study (including pig faeces and environmental samples) suggesting that transmission is likely (Salviati et al. 2015).

Nguyen and coworkers found the same plasmid types and gene variants of ESBL-producing *E. coli* in chickens and pigs suggesting that transmission from one livestock animal to another might be a risk that needs to be considered in biosecurity (Nguyen et al. 2021).

Another example where other livestock seems to impact the occurrence of ESBL-producing *E. coli* is the study of Dohmen and coworkers. This research showed that the presence of goats on pig farms increased the prevalence of ESBL-producing *E. coli*. It is controversial if the goats itself contribute to the spread of ESBL-producing *E. coli* or if goats are more frequent on farms with lower biosecurity standards which may be the cause of the increased prevalence of ESBL-producing *E. coli* (Dohmen et al. 2017a).

3.3. Factors influencing the spread of ESBL-producing *E. coli* within the farm

3.3.1. Production system

Farm management factors regarding the production system seem to also play a role in the transmission dynamics of ESBL-producing *E. coli*. The all-in-all-out (AIAO) swine production keeps groups of animals together, and when moved the previous stable is completely emptied. The protective effect of an all-in-all-out system was shown to be significant in a longitudinal study for ESC-R-EC conducted in Switzerland. The study included samples from 31 breeding pig farms (farrow to finish and multiplier farms, where piglets were transported to other fattening units) in 2018 and 2019. The proportion of farms positive for ESC-R-EC is shown in **Table 3** (Moor et al. 2021).

Table 3: ESC-R-EC positive and negative farms with different farming systems.

	all-in-all-out	Not all-in-all-out	total
Positive farms	0 (0 %)	5 (16.1 %)	5 (16.1 %)
Negative farms	15 (48.4 %)	11 (35.4 %)	26 (83.9 %)
Sampled farms (n)	15	16	31

Data taken from (Moor et al. 2021)

A different study combined a pharmacokinetic-pharmacodynamic (PKPD) model with an on-farm model of bacterial transmission using data from several published empirical studies and assessed different risk factors for ESBL-producing *E. coli*. Regarding farm types an all-in-all-

out system was compared to a continuous production system where pigs of different batches might be mixed. The model looked at two different outcomes, the prevalence of ESBL-*E. coli* positive batches of slaughter pigs at the end of the fattening period, and the percentage of pigs shedding more than $2 \log_{10}$ cfu/g. The assessment suggested the all-in-all-out system had a lower batch prevalence for ESBL-producing *E. coli* and lower faecal shedding rates in slaughter-aged pigs. The impact of the interventions slatted floor, solid floor, all-in-all-out and a continuous husbandry system were assessed in different combinations. As a baseline they used the average prevalence of ESBL-*E. coli* in batches, each consisting of a group of 40 slaughter-aged pigs coming from four different pens and the prevalence of animals being colonized at the time they go to slaughter over all batches. In all the scenarios (as regards different intervention measures) the farm concept was a breeder-finisher system. In the introduction scenario five colonized piglets were placed in one farm, either in one or different pens. In the baseline scenario pigs classified as “sick” could be treated with antibiotics but were kept in the same pen while in the model to determine the effectiveness of a sick pen the “sick” pigs were furthermore separated and placed in a separate sick pen. The main result of this aspect of the study is that one initial introduction of ESBL-producing *E. coli* into piglet pens may result in slaughter-aged pigs being colonized and testing positive for ESBL-producing *E. coli* for the duration a full production year. Results for the different other scenarios, addressing the floor type, comparing all-in-all out systems with continuous production and the impact of enhanced cleaning and disinfection are summarized in **Table 4**. Cleaning parameters for the baseline scenario were adopted from a Salmonella model assuming that *E. coli* act similarly (Hill et al. 2016).

Table 4: Estimated prevalence of ESBL-producing *E. coli* in slaughter-aged pigs including different farm factors and farm-interventions

Farm types and interventions	Prevalence of ESBL-producing <i>E. coli</i> positive batches ¹ of slaughter-aged pigs (5 th and 95 th percentile)	Percentage of pigs shedding over 2 log ₁₀ cfu/g of ESBL-producing <i>E. coli</i> (5 th and 95 th percentile)
<u>Baseline:</u> reflecting typical production systems in UK	39.4 % of batches (0.0–57.5)	18.7 % of pigs (5.9–30.4)
Continuous systems with slatted floor	34.4 % of batches (0.0–52.5)	22.0 % pigs (20.1–23.8)
Continuous systems with solid floor	48.1 % of batches (0.0–59.4)	26.4 % pigs (18.5–30.9)
AIAO housing with slatted floor	26.9 % of batches (0.0–51.6)	13.0 % pigs (6.9–15.2)
AIAO housing with solid floor	5.0 % of batches (0.0–56.3)	10.3 % pigs (5.1–14.4)
Enhanced cleaning and disinfection	6.3 % of batches (0.0–53.8)	7.2 % pigs (0.0–21.5)
Use of sick pens	0.0 % of batches (0.0–44.4)	0.1 % pigs (0.0–0.3)
Use of sick pens plus enhanced cleaning and disinfection	0.0 % of batches (0.0–47.5)	0.1 % pigs (0.0–0.3)

Data taken from (McCarthy et al. 2021)¹

¹ A batch of slaughter pigs is defined as a group of 40 finisher pigs from four pens going to slaughter

The positive effect of all-in-all-out housing on the prevalence seems to be easily explained by the fact that this farming system prohibits mixing of pigs of different production stages or origins and although it is not described in this study it usually allows a more thorough cleaning process of the stable between the new and the previous batch of pigs (Isomura et al. 2018, McCarthy et al. 2021). It should be considered that this assessment is the outcome of a complex modelling approach. Furthermore, the numbers taken as a base were retrieved from different studies with different background, countries and species.

A dutch study showed another effect of differences in production systems. Dohmen and coworkers did a longitudinal study during 2011-2013 where they sampled 36 farms. 60 pigs on each farm were sampled with rectal swabs. Beside the occurrence of ESBL-producing *E. coli* they also monitored the antibiotic usage during this time. Studying different conventional farm

types one result was that open farms used triple the amount of antimicrobials (measured in Defined Daily Dosages per Animal Year (DDDA/Y) compared to closed ones (Dohmen et al. 2017a). Open farms in this study were buying gilts from other farms at least annually. It could be assumed that this practice might lead to the transmission of germs and could potentially cause health issues in the herd leading to the need of a higher usage of antibiotics. This outcome points in the direction that closed farms might have less health problems and use less antibiotics due to not introducing new animals from suppliers into their herd. Independent the cause, the increased use of antibiotics in open farms should be considered when a concept for a farm is developed.

3.3.2. Transmission ways within a farm

A common way of transmission of resistant bacteria is direct contact to animals in or between compartments. In this context, it should be considered that there are different processes influencing the distribution of ESBL-producing bacteria in stables. Beside the mechanical contact where animals and humans (active) or stable utensils (passive) act as transmitter also physical mechanisms like turbulence and sedimentation need to be taken into account (Müller et al. 1977).

ESBL-producing *E. coli* have different ways of spreading. Besides the usual spreading of bacteria they also exchange resistance genes as well as plasmids carrying resistance genes (Sharp et al. 2014). Recent papers suspect that the resistance of *E. coli* (with a focus on cephalosporin resistance) is acquired through the spread of resistance genes which is supported by the selective pressure of antimicrobial substances rather than specific strains itself spreading through a population. Nevertheless, this horizontal spread of resistance genes seems to be quite effective because when detected in one compartment up to 100 % of the pigs in that compartment are colonized with the same strain. Schmithausen and coworkers considered compartments therefore as “hot spots” for transmission (Schmithausen et al. 2015b, Schmithausen et al. 2016).

Furthermore, an experiment on the effects of ceftiofur injections showed interesting effects on untreated animals in the same stable as the treated pigs. The test setup was that 6 healthy pigs (Group B) were treated with ceftiofur injections i.m. for the intended course of 3 days. Meanwhile a second equally big group of pigs (Group A) was held in the same stable in another compartment (1.6 meters apart with no direct contact). Both groups were tested negative for

resistant *E. coli* on the first day of the experiment. Beside the expected effect of the antibiotic on the commensal bacteria of the treated group, the use of antibiotics surprisingly also effected the untreated animals. 28 days after the treatment 3 animals of group A showed faecal samples with *E. coli* colonies being resistant to ceftiofur (MIC>8µg/ml) even though they have not been treated with antibiotics. Later in the study on day 42 (after one more treatment of group B with antibiotics) all pigs of both groups tested positive for ceftiofur-resistant *E. coli* regardless if they have been treated or not. PCR was used to identify resistance genes and showed that in the first test 10/11 and in the second test 8/12 tested *E. coli* had ESBL coding genes including TEM and CTX-M (Beyer 2014).

Hering and coworkers proposed that movement of animals inside of one stable may also contribute to spreading of ESBL-producing *E. coli*. This suggestion refers to the finding that stables with sick pens have a significantly higher prevalence of ESBL suspicious *E. coli* than the ones without a separate pen for diseased animals. One explanation of the study team for this phenomenon was that beside farms having these facilities might have more animals with health problems that they also transport sick pigs through the stable and therefore maybe spread resistant bacteria this way (Hering et al. 2014).

Air exposure seems to be also a possible route of transmission not just between but also within farms. Schmithausen and coworkers did elaborate research on the transmission of ESBL-producing *E. coli* on 35 pig farms. To take samples they used an air sampler at the height of 1.20 meters. Besides that, the pigs were tested on ESBL-producing *E. coli* using rectal swabs to look for a correlation with those in the air samples. All in all, they took 589 pig samples, 116 air samples and 367 dust samples. The results showed that 17 % of the pig farms had ESBL-producing *E. coli* in their air samples. Interestingly, in all farms where ESBL-producing *E. coli* were detected in the air samples it was also detected in the pig samples (rectal swabs) (Schmithausen et al. 2016).

In contrast to the 17 % positive farms by air samples they found higher frequencies of ESBL-positive air samples at the abattoirs. Out of the 12 samples they took from the abattoirs 6 were positive (50 %). Since the sample number was quite low, they just could describe a trend but could not interpret this data as a significant predictor. An interesting factor seems to be the humidity. Enterobacteriaceae require humidity on surfaces. Since the humidity of the air samples that were tested positive for ESBL-producing *E. coli* at the abattoirs was higher than those in the stable, this might be a relevant factor. In line with this hypothesis the ESBL-

transmission that was detected was higher at the abattoir at which the pigs were held in humid environment until slaughtering. Schmithausen and coworkers demonstrated that 29 % of the previously negative tested pigs acquired ESBL-producing *E. coli* in the waiting area of the abattoir (Schmithausen et al. 2015b).

Contrary to this, in a study on ESC-R-EC conducted in Switzerland out of 58 samples of settled dust of different stables only one (4.5 %) was found to be positive for ESC-R-EC. 16.1 % of the sampled farms were positive for ESC-R-EC and the ESC-R-EC prevalence of the pigs varied between 6.2 % and 1.8 % (See **Table 3**). All 60 air samples were negative for ESC-R-EC (Moor et al. 2021).

Anne Beyer and her team had two types of sampling methods for *E. coli*. First, they distributed native (without antibiotics) agar plates around the stable (near the pigs) to allow dust to settle there. As the second method they took samples from air filters. As previously described two pig groups, 6 animals each, were used for this experiment. While group B was treated with ceftiofur at the beginning of the experiment, group A remained untreated at first. Both sampling methods were able to detect *E. coli* in the first days of the experiment which then were shown to be susceptible to ceftiofur. At the end of the experiment plates near both pig groups and samples from air filters had cefotaxime resistant *E. coli* in them (most of them probably being ESBL-producing *E. coli*). It is interesting that although group A did not get any treatment and had no direct contact to the treated pigs in the first part of the experiment they had acquired resistant *E. coli* during the experiment (Beyer 2014). These findings give further evidence that the transmission of bacteria, especially of ESBL-producing *E. coli* over the air borne route needs to be considered.

Salviati and coworkers were able to detect ESBL/AmpC producing *E. coli* in air samples of pig barns. The sampling method were Glass-Impingers which were set up at a height of 1.50 m throughout the barns. Specifically, during the repeatedly sampled 7 pig fattening farms using enrichment methods they detected ESBL/AmpC producing *E. coli* in 6 out of 63 (9.5 %) samples that were from 4 different farms. When plated directly (without enrichment) in 4 of 63 (6.4 %) barn air samples they detected ESBL/AmpC-producing *E. coli*. Furthermore, dust samples were taken from places that the pigs had no direct contact with. They detected no ESBL/AmpC producing *E. coli* in any of the 21 dust samples (Salviati et al. 2015).

3.3.3. Level of farming intensity

A study on German livestock showed that the size of the stable and the sampled group had an impact on the prevalence of ESBL/AmpC producing *E. coli*. This finding leads to the conclusion that more intense contact of the animals leads to more transmission (Hille et al. 2018).

These dynamics are also shown by Salviati and coworkers. Out of the 7 tested farms the three farms with the highest number of animals per barn were the same three that had the highest occurrence of ESBL/AmpC producing *E. coli*. In contrast the farm with the lowest number of pigs per barn also was the one with the lowest occurrence of ESBL/AmpC producing *E. coli* (Salviati et al. 2014).

For beef as well as for dairy cattle, factors indicating a less intensive farm management were associated with lower numbers of cefotaxime-resistant *E. coli* samples (cefotaxime resistance in *E. coli* is often mediated by ESBL/AmpC genes (Friese et al. 2013)). For beef cattle, these factors were “only one stable for beef cattle”, “duration of fattening >18 months”, “feed: hay”, “cleaning of stables exclusively with pitchfork” and breed, i.e., “percentage beef Fleckvieh >50 %” (Hille et al. 2017). Less intensive farming seems to have a positive impact on the occurrence of ESBL-producing *E. coli*.

3.3.4. Husbandry

Since there is a high probability that ESBL-producing *E. coli* are transmitted through air and dust the exhaust air systems seem to be a factor influencing the transmission of bacteria. Underfloor ventilation has the reputation to be safer than overfloor ventilation because bacteria are directly sucked in and not distributed through the air. Nevertheless, Hering and coworkers found underfloor exhaust ventilation increased the risk for presence of ESBL suspicious *E. coli* significantly compared to overfloor ventilation. Although this effect is unclear one explanation might be that the dust from the surfaces of the stable is stirred up before being sucked into the system (Hering et al. 2014).

One promising intervention is the use of sick pens for the duration of treatment of sick individuals. In the British model, the use of sick pens reduced the calculated batch prevalence to 0.1 % (McCarthy et al. 2021). This effect might have several reasons: firstly, that the pigs which are currently not sick and not treated do not come in contact with antimicrobials which otherwise could lead them to develop ESBL-producing *E. coli* themselves. Secondly, they are

not in contact with the treated and sick pigs which might currently shed (more) ESBL-producing *E. coli* and pose a higher risk of transmission (see **Table 4**) (McCarthy et al. 2021).

However, another study on CREC/ESBL suspicious *E. coli* had contradictory results. Hering and coworkers found sick pens to have a significant negative impact on the prevalence of CREC. Farms with sick pens in their pig stables had a 2.8 higher mean count of positive samples for ESBL suspicious *E. coli*. In this case the scientists assumed that farms with sick pens might have more sick pigs in general and that the movement of the sick pigs is a risk for transmission of resistant bacteria through the stable (Hering et al. 2014).

Flooring does not just play an important role in the waste management system, but it also influences the animal comfort. Interestingly flooring seems to have different effects on the prevalence in different housing systems. While solid floor correlates with a higher prevalence of ESBL-producing *E. coli* in slaughter-aged pigs in a continuous system, it correlates with a lower prevalence in an all-in-all-out system. There are different explanations for this observed effect. Slatted floor allows faeces to be cleaned out faster, but after having fallen through the gaps the excretions are still in the stable environment and may still be a source of transmission. Additionally slatted floor might be harder to clean and disinfect than solid floor because of the uneven surface. The effects of flooring in pig stables in different farm systems needs to be further explored by more empirical research (see **Table 4**) (McCarthy et al. 2021).

When farms are sampled for research on ESBL-producing *E. coli*, the farmers are often asked to fill out a questionnaire on farm information, biosecurity measures and overall management systems. Ketkhaio and colleagues performed such a study on four pig farms in the region with the highest density of pig industry in Thailand. Farms A, B, and C were large farms and managed intensively, with more than 5000 fattening pigs. Farm D was a small farm with less than 50 fattening pigs and according to the study followed a more natural farming system. 347 samples were taken over a period of about 6 months. The samples included faecal swabs, boot swabs, floor swabs, clean water, and wastewater in variable numbers on each farm. To detect *E. coli*, the samples were cultured on coliform agar and water samples were cultured according to methods established for water quality control (International Organization for Standardization 2014). As a result, 206 *E. coli* were selected and further analyzed to identify MICs of different antimicrobials. Furthermore, they screened isolates for ESBL-production (Ketkhaio et al. 2021). The results showed that 35 (17 %) of the isolates were confirmed as ESBL-producing *E. coli*. The majority of ESBL positive samples were from Farm A and C, with

15 (n=43, 34.9 %) and 11 (n=52, 21.2 %) positive samples respectively. In contrast Farm D had only 6 (n=55, 10.9 %) ESBL-positive isolates while Farm B only had 3 (n=56, 5.4 %) positive isolates (Ketkhao et al. 2021).

In the questionnaire, the following biosecurity measures were looked at: fencing, boot separation/changing boots, access to the farm (restricted/not restricted), pest control, separated workers for each swine housing, boot cleaning before and after entering housing, outside vehicle cleaning before entering to the farm and manure system. Regarding the antimicrobial use the noted farm practices were “feed medicated for nursery swine” (different antibiotics) and “therapeutic treatment (injection)”. To see if the biosecurity measures and farm management factors had any effect on the prevalence of ESBL *E. coli*, the farm practices of the two farms with the lowest ESBL-producing *E. coli* isolates (Farm B and D) were studied to find similarities (**Table 5**). The following practices were the same on farm B (5.4 % ESBL-producing *E. coli* isolates) and D (10.9 % ESBL-producing *E. coli* isolates) contrary to Farm A (34.9 % ESBL-producing *E. coli* isolates) and Farm C (21.2 % ESBL-producing *E. coli* isolates): no manure system and no changes of clothes. However, more importantly Farm B and D were the only ones that used no medicated feed for nursery swine, and when treated with injections Farm B used just one antibiotic (Gentamicin) and Farm D none. Contrary to that, Farms A and C used several types of antimicrobials in feed for nursery pigs as well as therapeutic injections (Ketkhao et al. 2021). Since there are no definitions provided for the measures, it remains unclear what some interventions like “separation/changing boots” or “No manure system” exactly mean.

Table 5: Comparison of farm practices of farms with a lower (farm B and D) and a higher (farm A and C) ESBL-producing *E. coli* prevalence

Factors that were the same in Farm B and D (only)	Factors that were the same in Farm A and C (only)
No manure system	Covered lagoon
No clothes changing	Clothes changing (farm C), wearing white coat (farm A)
No medicated feed for nursery swine	Feed medicated for nursery swine (including Amoxicillin as a β -lactam)
Less antibiotic classes for therapeutic treatment (injection): Gentamicin in farm B and None in farm D	Therapeutic treatment (injection) with several antibiotics: including Ampicillin (farm A) and Ceftriaxone (farm C) as β -lactam

Data taken from (Ketkhao et al. 2021)

3.3.5. Cleaning and disinfection

Different hygiene measures have different effects on the occurrence, survival and transmission of bacteria. Therefore, hygiene, cleaning and disinfection are important measures when it comes to managing resistant bacteria. The reason for this is that better hygiene controls the transmission of infectious agents, e.g. bacteria (Schmithausen et al. 2018). This results in fewer infections to treat with antibiotics and therefore the selective pressure on bacteria by antibiotics is reduced. At the same time the transmission of already resistant bacteria can be prevented. At cattle farms it has been shown that there was a correlation between cleaning of the stables and the number of cefotaxime resistant *E. coli*-positive stables. A higher frequency of cleaning correlated with less positive samples. Other hygiene indicators that correlated with a lower detection rate for cefotaxime-resistant *E. coli* in the cattle farms were “use of boots for one stable only” and, “control of flies with traps” (Hille et al. 2017). Overall farm biosecurity and especially cleaning and disinfection play an important role in controlling of AMR (Davies and Wales 2019). The quality of cleaning and disinfection also seems to have a big impact since recently cleaned and disinfected pens have been shown to be still partially contaminated with AMR *E. coli*. The stable environment such as drinkers also prove the potential to be a source

of infections with antimicrobial resistant bacteria. Even cleaning tools tested positive for ESBL-producing *E. coli* at the first sampling as well as 11 months later (Lucia et al. 2021).

In the study of Gay and coworkers “soak the floor” resulted to be a risk factor for ESBL producing *E. coli*. Gay and coworkers suggested that this could be due to false biosecurity practices such as soaking for a too short period of time. “Detergent use for cleaning” turned out to have a protective effect by lowering the occurrence of ESBL-producing *E. coli* (Gay et al. 2018). In a different model including just the regions Reunion, Mayotte and Madagascar the only significant risk factor was “change shoes/boots before entering house/pen”. Although the reasons for that have yet to be investigated, one theory is that more modern farms practice this technique which in some cases also tend to be the farms that use more antimicrobials. As protective factors in this model they discovered “rodent control by a company” and “two disinfections between two consecutive batches” (Gay et al. 2018).

Looking at hygiene indicators, the farms that emptied their manure pit after each batch only, had higher rates of MDR bacteria than the ones that emptied it every week. The reason for this is possibly that the more frequently the pit is emptied the less MDR *E. coli* are present to infect the animals or circulate among the animals. Surprisingly the farms that never cleaned and disinfected their finisher pens between two consecutive batches of pigs had a lower risk to have MDR bacteria in their samples. Since this answer was only given by breeder-finisher farms the reason for that could be the continuous production conditions and not actually the cleanliness. Another reason for this finding may be the fact that disinfectants, if used incorrectly by diluting them too much, can cause bacteria to become resist against disinfectants (AbuOun et al. 2020).

According to the sensitivity analysis in the UK model, old faeces and the timeframe they were cleaned out played a big role in the overall prevalence (McCarthy et al. 2021). If put into perspective this factor is a hygiene indicator and is connected to cleaning out of the stable and to the kind of floor the pigs are kept on. Advanced hygiene, in this case enhanced cleaning and disinfection, showed a reduction of batch prevalence to 6.3 %, which is less than the calculated baseline of 26.9 % in all-in-all-out system on slatted floor (**see Table 4**).

3.3.6. Pest control

Rodents are found to be vectors and potentially spread antimicrobial resistant bacteria. Among three samples from mice collected at one pig fattening farm Salviati and coworkers were able to detect ESBL/AmpC-producing *E. coli* in one sample (33 %) (Salviati et al. 2015). Dohmen and coworkers found the measure “pest control by specialists” to have a protective effect by significantly decreasing the prevalence of ESBL-producing *E. coli* (Dohmen et al. 2017a). Rodent control is an important measure to reduce the risk of rodents spreading ESBL-producing *E. coli*. Researchers in Réunion found the measure “rodent control by a company” to be preventive for ESBL-producing Enterobacteriaceae (the majority in this study being *E. coli*) in pigs correlating with a p-value of 0.01 (Gay et al. 2018). Other research was not as successful at proving mice as ESBL-producing *E. coli* carriers. In Switzerland 17 samples of mouse faeces were tested for ESC-R-EC. The samples were taken at the first visit (whenever faeces were found) from the floor. Ten of the samples were from maternity units, while the other 7 were taken from fattening units. The study does not say which farms the mouse samples were from, but since on 12 of 31 sampled farms ESBL genes were present there is a chance that some of the mouse faeces were from positive farms. In this study none of the mouse faeces showed contamination with ESC-R-EC (Moor et al. 2021).

Other vermin like flies are also a possible transmission route for ESBL-producing *E. coli*. 53 flies were taken on several occasions from 31 different pig farms in the same study. When tested for ESC-R-EC two (3.8 %) of them carried ESC-R-EC (Moor et al. 2021). Salviati and coworkers also were able to show the ability of flies carrying ESBL/AmpC producing *E. coli*. Three out of 12 (25 %) fly samples were positive for ESBL/AmpC producing *E. coli* in this study. The positive samples were taken from 6 of the 7 farms (Salviati et al. 2015). Research on flies had shown before that they can carry ESBL-producing *E. coli* (although this study was on other livestock, not pigs) (Poudel et al. 2019). Toxins can be used as fly control to prevent transmission of resistant bacteria. This measure showed to have a significant effect on the reduction of the prevalence of ESBL suspicious *E. coli* in German pig farms compared to no fly control (Hering et al. 2014). As mentioned before, Hille and coworkers found the factor “control of flies with traps” to correlate with less cefotaxime-resistant *E. coli* infection in cattle farms (Hille et al. 2017).

3.3.7. Antibiotic usage and application route

There are different ways how livestock is treated with antibiotics. Antimicrobial substances can be used prophylactic, metaphylactic or tailored to an indication. In many countries antibiotics are also used as growth-promoters in feed. Growth promotion is not allowed anymore in the EU and use of antimicrobials for prophylactic purposes is quite restricted (Regulation 2019/6 Article 107).

Gay and coworkers found the factor “recent antibiotic use (<1 year)” to be a risk factor for the occurrence of ESBL producing *E. coli* (Gay et al. 2018).

Besides the use itself another factor influencing the impact on resistance is the duration of antimicrobial use. It has been proven that long-term use compared to short-term therapies with antibiotics is a predictor of resistance. This was shown by Gibbons and coworkers for trimethoprim/sulfamethoxazole. Furthermore, it was shown that the use of aminoglycosides, tetracyclines and trimethoprim/sulfonamides was associated with increased odds of gentamicin, tetracycline and trimethoprim/sulfamethoxazole resistance respectively. In addition, there was an indication that the use of trimethoprim/sulfonamides led to resistance to other antimicrobials, in particular to ampicillin, streptomycin and tetracycline. This phenomenon might be explained by co-selection and co-resistance. There were even findings that β -lactam antibiotics decreased the odds of resistance to trimethoprim/sulfamethoxazole (Gibbons et al. 2016). The findings might differ depending on which antimicrobial is used. A study from 2015 detected only resistance to quinolones (ciprofloxacin, moxifloxacin) and found that the use of these antibiotics on the farms did not correlate with the resistance patterns (Schmithausen et al. 2015b). Dohmen and coworkers found that not the antibiotic usage itself but more so the antibiotic class impacted the occurrence of ESBL-producing *E. coli*. The use of cephalosporins was significantly associated with the farms being positive for ESBL-producing *E. coli* (Dohmen et al. 2017a).

Another example of the effects of antimicrobial use over a long period of time is a Danish study on the prophylactic use of ceftiofur. The resistance gene CTX-M-1 mediating resistance to β -lactam antibiotics was found in many samples. In the faecal samples 71.4 % (50/70) of the tested pigs were positive for CTX-M-1 *E. coli*. Furthermore, the same resistance gene was found in three of the four tested humans having contact with the pigs indicating a transmission to people. In terms of resistance the isolates showed a particularly high rate (80 %) of resistance to two or more non- β -lactam antibiotic classes (Moodley and Guardabassi 2009).

This study emphasizes the high impact of cephalosporins on the resistance patterns of *E. coli* especially if used prophylactically.

One other concern is the form of application of antibiotics. While injections are very specific regarding the dose as well as the specific individual, the application via feed is not. Moreover, sick animals often tend to eat and drink less which makes underdosing more likely. Underdosing, whether another animal is eating some of the left antibiotics or the treated animal itself is getting too little, could promote even more selection of resistant bacteria (Hille et al. 2017). It seems that the different practices of how antimicrobials are used have different impacts on the prevalence of ESBL. The difference between selected targeted treatment and treatment of whole groups of animals is also connected to the prevalence of ESBL-producing *E. coli*. A study on cattle showed that antimicrobial treatment of “all animals” in one group had a significant correlation with more positive samples of cefotaxime-resistant *E. coli* while the group where only the sick individuals were treated did not. In this study the effect of single animal treatment via injections did have no significant impact on the occurrence of cefotaxime-resistant *E. coli* (Hille et al. 2017). A similar study on pigs showed that group treatments multiplied the odds of a farm to be ESBL-producing *E. coli* positive by 4. The definition of farms using group treatment in this study was that $\geq 50\%$ of the treatments were administered to the whole group instead of individuals. The antibiotics used in this case were tetracyclines, penicillin, trimethoprim, macrolides, polymyxins and others (Dohmen et al. 2017a).

Furthermore, Anne Beyer suggested that subtherapeutic doses in the environment (for example in dust or aerosols) may contribute to the development of resistant bacteria. Her study showed minimal ceftiofur concentrations in aerosols and dust samples even though the animals were treated by injection. In this scenario underdosing seems relevant not only for medicated pigs but also people working with them and might inhaling residual antimicrobials (Beyer 2014). In the experiment, as previously described they had two groups of pigs. It is interesting that the groups reacted very differently to their first ceftiofur treatment. While the group that was treated at first showed a slight increase in resistant *E. coli* the group that was treated after 45 days in the same stable as the already treated group showed a significant increase in resistant colonies. The researchers explain this effect by the theory that the second group had contact to subtherapeutic doses prior to their first treatment due to transmission through air and dust. This favored the selection of resistant *E. coli*. After their first treatment the already resistant bacteria increased heavily (Beyer 2014).

Conversely, a Canadian study comparing different husbandry conditions found the antibiotic free system to be the one with the most ESBL phenotypes compared to conventional husbandry. This surprising finding might be explained by prior antibiotic use which the researchers were unable to find detailed information on (Poulin-Laprade et al. 2021).

3.3.8. Animal health management

Other factors such as cold stress seem to increase the prevalence of resistant *E. coli*. Specifically the increase in ampicillin and tetracycline resistant *E. coli* could be noted, in the research of Moro and coworkers, when the temperatures declined below the zone of thermoneutrality (Moro et al. 1998). One explanation for that could be the changing feed intake due to the different temperature which might alter the gastrointestinal microflora. Weaning might be a similar stressor and could have the same effects on microbiome (Moro et al. 1998).

The overall health of pigs seems to influence the occurrence of ESBL-producing *E. coli*. ESBL-producing *E. coli* possibly correlate with other pathogens. The simultaneous colonization of ESBL-producing *E. coli* and Methicillin-resistant *Staphylococcus aureus* (MRSA) was found in 9 % of the pigs in the study of Schmithausen and coworkers. The risk of being colonized with MRSA was 3,25 times higher for pigs that are also colonized with ESBL-producing *E. coli* and the other way around. In these particular farms, tetracycline and β -lactam antibiotics have been used and are probably a factor influencing occurrence and horizontal spread of the ESBL-producing *E. coli*. Overall ESBL-producing *E. coli* and MRSA seem to be good indicators for the health status of herds and antibiotic use of the farms (Schmithausen et al. 2016).

A study on the post-weaning piglet diarrhea showed a significant connection between virulence genes and antibiotic resistance. Post-weaning piglets were more prone to diarrhea. Furthermore, they had *E. coli* harboring significantly more virulence genes and more ESBL-producing *E. coli*. The research team collected 909 faecal samples from organized farms in India. They found 531 *E. coli* isolates on selective cefotaxime agar. 345 isolates of them were ESBL-producing *E. coli*. Overall they showed that diarrheic samples had significantly more antimicrobial resistant isolates (VinodhKumar et al. 2019).

3.4. Characteristics of ESBL-producing *E. coli* carriage

3.4.1. Production stage

E. coli isolates show the highest resistance rates when taken from earlier stages (grower pigs) of production, while gilts, sows and finisher pigs have less often resistant *E. coli*. Finisher pigs show the lowest levels of multiresistance (Moro et al. 1998). In the study of Gibbons and coworkers the stage of production correlated significantly with the prevalence of resistance to ampicillin, streptomycin, trimethoprim/sulfamethoxazole, tetracycline, chloramphenicol and gentamicin (Gibbons et al. 2016). As mentioned Moro and coworkers showed that cold stress increases the level of resistance to ampicillin and tetracycline significantly (Moro et al. 1998). Gibbons and coworkers suggested therefore that the stress of weaning acts as a similar stressor on the pigs and is the reason for the increased resistance rates at this production stage (Gibbons et al. 2016). Another reason may be the fact that since weaner pigs are more often exposed to enteric diseases, they also get treated with antibiotics more often. The stress from weaning, regrouping and contact with new pigs may lead to more frequent transmission of resistant bacteria as well as susceptibility to infections (Akwar et al. 2008). Pigs who get closer to the ready-to-slaughter stage seem to have less resistant bacteria and the amount of detected ESBL-producing *E. coli* is declining. One explanation for that could be the restricted antibiotic usage at this production stage (Schmithausen et al. 2015b).

3.4.2. Carriage duration

A risk assessment from 2021 involving a PKPD model using data from several published empirical studies showed new insights into the transmission dynamics of ESBL-producing *E. coli* in pig farms. When simulating transmission of ESBL-producing *E. coli* through infected piglets the study showed that colonization with these bacteria lasts for at least one year on commercial farms. The calculation showed a steady plateau of the prevalence on the farm about 23 weeks after introduction of the new type of resistant bacteria. Both pathways, introducing five positive piglets to the same pen, or to several pens led to the spread and persistence of the resistant bacteria (McCarthy et al. 2021).

Carriage of ESBL-producing *E. coli* seems to fluctuate during a pig's life. Moor and coworkers did a longitudinal study on 31 pig farms. One of the results was that the carriage duration of ESC-R-EC of individual pigs is quite short term. Of the 57 pigs that carried ESC-R-EC during

the suckling phase, 32 (57.1 %) did not test positive for ESBL-producing *E. coli* in the weaning period (Moor et al. 2021).

3.5. Selective pressure on ESBL-producing *E. coli*

3.5.1. Antibiotics

The evolution/selection of AMR was tested in a Danish study on the swine faecal microcosmos. The study of Herrero-Fresno and Olsen focused on faecal samples and the response of resistant coliforms to beta-lactam antibiotics. They mention that these coliforms were most likely *E. coli*, however they did no specification. Since the tested antibiotics were two kinds of beta-lactam antibiotics and cephalosporins they assumed that ESBL played a role in the occurrence of resistance. The study did not specifically test for ESBL-genes or β -lactamases. Herrero-Fresno and Olsen took faecal samples of 32 nursery pigs of four different farms. By mixing them two by two (only samples from the same farms were mixed) they received 16 samples to be tested. The samples were treated with ampicillin, cephalexin, ceftiofur and tetracycline (to see the effect of one other antimicrobial beside beta-lactam antibiotics). For the baseline the samples were 10-fold diluted in Dulbecco's Phosphate Buffered Saline (DPBS). Next, they were plated on McConkey agar with and without antimicrobials (2x breakpoint concentration (BPC) and 4x BPC). After 24 hours the colonies which were suspected to be coliforms were counted to find out the proportion of resistant coliforms (T0h). Colonies that grew on plates fortified with antibiotics were generally classified as resistant without further testing. To determine the change in resistance after treatment with antibiotics each sample was divided into five samples which were each treated with one of the mentioned antibiotics. One control sample was left untreated. Concentrations of antibiotics similar to the amount reaching the gut when treated with usual doses were applied. After 6 hours of incubation (T6h) they followed the same procedure as at T0 to determine the proportion of resistant colonies (dilution and plating on McConkey fortified with and without AB). For the regrowth factor after the treatment the samples were diluted 1:100 with DPBS and further incubated for 20 hours (T20h). Repeating the same procedure, the samples were then diluted and plated. After 6 hours of exposure to the antimicrobial the proportion of resistant coliforms had increased significantly. Interestingly, the resistance at T20h depended on the drug. Colonies treated with first and third-generation cephalosporins had a higher proportion of strains with multidrug resistance (Herrero-Fresno and Olsen 2020). This study gives evidence that different drugs

exert different selective pressure and therefore may be more prone to induce AMR. The results indicate that also short treatment has a severe impact on the occurrence of AMR.

The work of Bergšpica and coworkers showed another correlation between antibiotic usage and the occurrence of resistant *E. coli*, supporting the hypotheses that antibiotics promote resistance in *E. coli* isolated from pigs. Bergšpica and coworkers found a medium level of correlation between the penicillin sales for livestock and the prevalence of *E. coli* suspected to be ESBL-producing in the EU in 2017 (Bergšpica et al. 2020). Farms using a moderate amount of antimicrobials showed to have nearly three times the odds to have pigs being positive for multidrug resistant *E. coli* compared to farms with low usage of antibiotics ($p=0.05$). Even more drastic, farms with a high amount of antibiotics in their treatment regime had an even 5 times higher likeliness to have multidrug resistant *E. coli* in their animals ($p<0.001$). The farms were categorized subjectively on the amount of antibiotics that were given per animal per day and the costs of antibiotics per year (AbuOun et al. 2020). *E. coli* that were retrieved from a ciprofloxacin enrichment plate (being ciprofloxacin resistant) had generally more AMR genes than the ones retrieved from antibiotic-free plates. *E. coli* that are therefore resistant to ciprofloxacin have acquired more other AMR genes which points to co-selection playing a role in this process. Foreseeable the more antibiotics were used on the farms the more AMR was found (AbuOun et al. 2020).

Analysis of data collected through a questionnaire on farm information, biosecurity measures and overall management systems showed several correlations between husbandry, antibiotic regimes and the prevalence of ESBL-producing *E. coli*. As already mentioned, there is a possibility that the effects were mainly influenced by the differences of the antibiotic usage. The farms that had a higher prevalence of ESBL-producing *E. coli* used several antibiotics including beta-lactams and used medicated feed for nursery pigs (**see Table 5**). In comparison, the farms with the lower prevalence of ESBL-producing *E. coli* did not use medicated feed and the antibiotic injections included less antibiotic classes (Ketkhao et al. 2021).

3.5.2. Other substances with potential influence on AMR

Since the ban of antibiotics as growth promoters in 2006 in the EU a lot of alternatives to help pigs grow were marketed. As post-weaning diarrhea is one of the biggest health-problems in young pigs a lot of alternative approaches were used in the pig-industry such as organic acids, bacteriophages, probiotics, herbal products as well as trace elements like zinc oxide. Zinc

oxide as a feed additive has gotten quite popular and has been frequently used since the ban of antibiotics as growth promoters. The dosage in which zinc is needed in young pigs to promote normal health is 80-100 mg/kg in their diet (Ciesinski et al. 2018). The EU restricted the maximum content of zinc in feedingstuff to 150 mg/day or 150 mg/kg feedingstuff (Regulation (EC) No 1334/2003). Nevertheless, zinc supplements as veterinary products were allowed to be used in higher amounts up to 2500 mg zinc oxide/kg diet if only used for 14 days up to the year 2022. Zinc supplements have a positive effect on post-weaning diarrhea (Case and Carlson 2002). A recent study found negative effects of high dietary zinc on resistance patterns in *E. coli*. 32 piglets were divided in two groups and fed with different amounts of zinc. While the first group got 2103 mg/kg diet zinc, the control group was fed with 72mg/kg diet zinc. The high zinc diet was fed over the course of 4 weeks. Although this is longer than formerly allowed for the veterinary products the authors argued that this practice is common. Samples of faeces, mucosa and digesta were collected at different time points. In this study the resistance was tested with agar plates fortified with different antimicrobials which were evaluated visually for colonies after the samples were plated and incubated. The antimicrobials tested were ampicillin, enrofloxacin, sulfamethoxazole-trimethoprim and tetracycline. While the samples taken shortly after weaning did not show a clear tendency of the effects of the zinc concentration in the diet, the last samples taken at 52 days post weaning showed significant effects in all three sample groups (see **Table 7**). The multiresistant isolates were significantly higher in the high zinc group than in the control group in the samples obtained from faeces, mucosa and digesta. Interestingly, shortly after weaning the multi-resistant isolates were higher in both groups (Ciesinski et al. 2018). This aligns with the theory that weaning itself, acting as a stressor, causes a rise in resistant *E. coli* (Gibbons et al. 2016). Surprisingly the percentage of resistant isolates of the digesta samples at 38 days was significantly higher in the control group (see **Table 6**). It seems that the high zinc feed did not impact the overall count or resistance of *E. coli* but more so prevents the following decrease in resistance. This finding cannot be generalized for the whole gastrointestinal tract since the samples were just taken from the ileum (Ciesinski et al. 2018).

Table 6: Prevalence of multiresistant *E. coli* isolates (3 or more different antimicrobial classes) at the age of 38 days

	faeces	digesta	mucosa
High zinc group	31 %	36.2 %	43.1%
Low zinc group	35.4 %	49.7 %	45.5 %
Significant difference/significance	no	High/yes	no

Data taken from (Ciesinski et al. 2018)

Table 7: Prevalence of multiresistant *E. coli* isolates (3 or more different antimicrobial classes) at the age of 52 days

	faeces	digesta	mucosa
High zinc group	29 %	30.2 %	28.9 %
Low zinc group	14 %	7.2 %	5.8 %
Significant difference/significance	high/yes	high	high

Data taken from (Ciesinski et al. 2018)

A Portuguese study sampled two farms with different antimicrobial regimes against postweaning diarrhea. Both farms used zinc oxide prophylactically at a concentration of 0.15 % (=1500 parts per million (ppm)) in feed as a supplement. Farm A previously used colistin as antimicrobial but this practice was discontinued about two years prior to this sampling. Furthermore, farm A used amoxicillin unregularly as a treatment for diarrhea. There is no data on the dosage or frequency in which amoxicillin was applied. 102 faecal samples (rectal swabs) were analyzed and showed a high frequency of ESBL-producing *E. coli*. 62 ESBL-producing *E. coli* isolates were collected from both farms, the majority producing CTX-M-15. Farm A had a higher count of 35 ESBL-producing *E. coli* isolates than Farm B with 27 ESBL-producing *E. coli*, respectively. The higher level of ESBL-producing *E. coli* in farm A could also be related to their use of amoxicillin (Fournier et al. 2020). In the study, it remains unclear how many samples or pigs have been positive for ESBL-producing *E. coli* as only the number of isolates is reported. The researchers did not discuss the potential impact of zinc usage as prophylaxis.

It is possible that the high detection rates for ESBL-producing *E. coli* rates are connected to this practice.

Peng and coworkers did some research on the influence of zinc oxide on ESBL-producing *E. coli*. Pig faeces were collected from farms that have not been using antibiotics or zinc in their production systems. Two *E. coli* strains were selected for the experiment, one carrying the resistance gene *bla*_{CTX-M-1} on IncI1 resistance plasmid (MG1655/pTF2), while the other one carried the gene on the chromosome (MG1655::*bla*_{CTX-M-1}). Both were placed in a microcosmos with increasing zinc concentrations. The other coliforms beside the two selected *E. coli* strains reacted to the high zinc concentrations by significantly decreasing at high concentrations. The strain with the resistance gene on the plasmid (MG1655/pTF2) showed a higher proportion under the influence of high zinc concentrations in comparison to the total flora. Interestingly the strain carrying the gene on the chromosome (MG1655::*bla*_{CTX-M-1}) did not show the same shift in proportions. This finding suggests that the growth advantage of zinc on *E. coli* carrying resistance genes may be limited to strains carrying the IncI1 plasmid. Both selected *E. coli* strains showed significantly increased levels of *bla*_{CTX-M-1} mRNA grown under the influence of zinc oxide in comparison to no zinc addition. This may reflect induction of expression of resistance genes (Peng et al. 2020).

A new approach against antibiotic resistance is to use polyphenols as a natural source of antimicrobials. Polyphenols, in this case phlorotannins, are extracted from brown seaweeds. *Fucus serratus* and *Ascophyllum nodosum* were used to harvest these extracts. The MIC of phlorotannin extracted from *A. nodosum* against *E. coli* was found to be the lowest with 0.781 mg/ml. Furthermore the study showed increased viability of intestinal porcine epithelial cells (Ford et al. 2020).

There is new research regarding Essential Oils as antimicrobial active substances. Găliņa and coworkers did a small study on 10 *E. coli* isolates from pigs. The included *E. coli* isolates were ESBL producers, MDR or susceptible to the tested antimicrobials. The most promising result was *Thymus vulgaris* combined with Sodium chloride (NaCl). This combination reduced ESBL-producing and MDR *E. coli* significantly. Since the sample group was very small there is not enough evidence in this field. Furthermore, there are concerns that Essential Oils may also lead to antimicrobial resistance (Găliņa et al. 2022).

3.6. Measures to reduce ESBL-producing *E. coli*

3.6.1. Measures to reduce antibiotic use

The increase of antibiotic resistance led the Dutch government in 2008 to implement different measures to promote prudent use of antibiotics in animal husbandry. The Taskforce of Antibiotic Resistance in Animal Husbandry was formed including members of different parties of the animal production chain and government departments. This group developed action plans for each sector of animal production. The aim of these plans were to increase the monitoring of not only the antimicrobial use and antimicrobial resistance but also to clarify the responsibilities of farmers and veterinarians regarding prudent use of antibiotics (Speksnijder et al. 2015).

In 2010, an independent organization called “Netherlands Veterinary Medicines Authority” was set up. This organization monitors antimicrobial use and prescription from farms and veterinarians. Based on these data, they were able to set up benchmarks to characterize three groups according to their level of antimicrobial use. By implementing a regulation, the Veterinary Medicines Authority was able to impose sanctions to the group with the highest AMR use.

These measures resulted in a decrease of antibiotic use among most livestock species. They showed that the decrease of antibiotics used in 2012 was 10-17 % compared to 2011. This reduction was measured in the unity “*defined doses per animal year*” (Speksnijder et al. 2015). When looked at the period of 2007 to 2012 the Veterinary Medicines Authority registered a decrease of 56 % in antimicrobial use in livestock. Beside the overall reduction of the total amount of antibiotics used in farm animals they also saw a drastic decrease in use of critically important antimicrobials (in this case 3rd and 4th generation cephalosporins and quinolones) (Bondt et al. 2012, Speksnijder et al. 2015). Although the effect of this reduction of antimicrobial use on resistance is hard to measure, in the following years a trend of decreasing resistance levels in *E. coli* in slaughter pigs was noted (MARAN 2013). As a monitoring program in the Netherlands indicator *E. coli* are randomly isolated of different animal species. 2019 was the first year since 1999 in which no ESC-R-EC were detected in indicator *E. coli* in faecal samples. In the following year one slaughter pig was positive for ESC-R-EC. However, using selective methods the prevalence in ceacal samples of pigs was still 9.9 % (Greeff et al. 2020, 2021). This leads to the conclusion that although ESC-R-EC were decreased in the last years they are still present in small concentrations (Greeff et al. 2020).

Danish authorities also took actions against the increasing numbers of ESBL-producing *E. coli* in 2010 and enforced a voluntary ban of cephalosporins in the pig industry. Agersø and Aarestrup were also able to show a significant decrease of extended-spectrum cephalosporinase-producing *E. coli* in slaughter pigs and on farms in Denmark in 2011 compared to 2010. There is an ongoing trend in finding an overall reduction in the resistance proportion in slaughter pigs (Agersø and Aarestrup 2013).

A long-term study in France was able to document the trends in antimicrobial resistance over a 14-year period. They were able to look at the effects the public awareness and regulations had on antimicrobial resistance. In 2010 the topic got more attention and there were efforts to decrease the amount of antibiotics used in the animal production and recommendations for veterinarians and farmers by the French Directorate for Food were published. The trends of resistant *E. coli* in pigs showed a peak for all tested antibiotics in 2010 and 2011 and started to decrease in the following years. Although this has not yet been formally assessed, it is reasonable to assume that the reduction in AMR is a consequence of the measures to reduce antibiotics in livestock. More studies on the influences of measures and antibiotic use on AMR are needed (Boireau et al. 2018).

A Japanese study was able to show a different approach to animal health and high production levels than using antibiotics. When they connected the different measures of animal welfare, biosecurity and antibiotic usage they could see a clear relationship. The farms that had better external biosecurity and a better location (regarding the distance to other farms and abattoirs) did use less antibiotics. Meanwhile they could not find any correlation between pig welfare or internal biosecurity and the amount of antibiotics that were used (Isomura et al. 2018).

How is it possible to reduce the amount of resistance when resistant bacteria have already spread on a farm? De Lucia and coworkers conducted a study on a farm in the UK that has been administering antimicrobial group treatment since the 1990th. Since the farm had ongoing problems with salmonellosis, whenever gastrointestinal problems in weaned pigs occurred, medicated feed was given to the whole group (not just the clinically ill pigs). Antibiotics administered to the pigs were apramycin (APR) and trimethoprim-sulfamethoxazole (SXT). At the first visit of the researchers in 2016 this practice was discontinued and instead only the clinically ill animals were treated (with the same antibiotics as before). During the three visits in May 2017 (T1), September 2017 (T2) and April 2018 (T3) samples were collected from weaners, growers and finisher pigs. Beside faecal samples, also environmental samples were

taken. For enumeration of *E. coli* colonies, the samples were pooled, diluted and plated onto agars containing no antimicrobial, trimethoprim-sulfamethoxazole and apramycin respectively. *E. coli* were identified by their chromogenic properties and after subculturing for purity verified by MALDI-ToF MS. The isolates were categorized into non-wild type (having a minimum inhibitory concentration above the defined epidemiological cut-off) and wild type. In all 53 pooled pig faecal samples indicator *E. coli* and SXT resistant sub-populations were detected. Beside four finisher samples all samples additionally contained APR resistant *E. coli*. De Lucia and coworkers were able to show a significant increase in indicator *E. coli* isolates being susceptible to all tested antibiotics (completely susceptible) between the first and last sampling moment as a result of the decreased antibiotic use. Different *E. coli* populations from plates retrieved with and without antibiotics also changed their proportions of wild-type and non-wild type between the first and last visit (see **Table 8**). The β -lactamase genes mediating resistance were bla_{tem} and bla_{oxa} (Lucia et al. 2021).

Table 8: Changes in proportion of wild-type (susceptible) *E. coli* to different antibiotics between T1 (shortly after reduction of antibiotic use) and T3 (two years after reduction of antibiotic use) – just the antibiotics that showed significant changes are listed (ciprofloxacin and florfenicol are not displayed)

Antimicrobial	Indicator <i>E. coli</i>	<i>E. coli</i> from APR-plates	<i>E. coli</i> from SXT-plates
Ampicillin	Significant increase	Significant increase	Increase
Trimethoprim-sulfamethoxazole	Significant increase	Significant increase	Increase
Apramycin	Increase	Decrease	Decrease
Tetracycline	Increase	Significant increase	Decrease
Streptomycin	Increase	Significant increase	Significant increase

Data from (Lucia et al. 2021)

The reduction of antimicrobial resistant isolates might be caused by the little selective advantage it gives *E. coli* when the antimicrobial treatment is stopped. In conclusion it is possible to reduce the prevalence of resistant bacteria to different antibiotics by a decreased frequency of antimicrobial treatment, but the time frame needed to observe any trend might be different for each antimicrobial. Although there was a reduction in the proportion of resistant *E. coli*, there was no elimination of the resistant bacteria (Lucia et al. 2021). In conclusion it is hard to eliminate resistant bacteria without a radical decontamination nevertheless, a reduction of resistance could be achieved.

3.6.2. Measures to reduce ESBL-producing *E. coli*

There is a lot of evidence on risk factors of ESBL-producing *E. coli* based on the correlation of factors and prevalence. Some authors make suggestion which measures could be beneficial

to eliminate these risk factors. There is little evidence on implemented measures in pig farms and their consequences. This lack of experiments might be due to the high financial risk that needs to be taken when a change is implemented at a farm. It would be even more ideal if two identical farms or two groups of pigs on one farm would be used to have a control group present. These requirements were met by few studies. In the following passage we included the research where effects of a change in the regimen were studied.

3.6.2.1. Decontamination

The question is what to do if ESBL-producing *E. coli* are present on a farm. Schmithausen and coworkers tried a radical decontamination on a farm where ESBL producing *E. coli* (and MRSA) were detectable in pigs. The decontamination process was carried out by a certified company over the course of one month. During that process all technical installations were removed, and new ones were installed afterwards. An elaborated cleaning process and disinfection was performed in the two previously used stables. This included high pressure cleaning, foaming, the use of a detergent and disinfection. During and after the procedure the regulations for visitors were intensified. Contact to pigs two days prior to the visit of the stable was prohibited. The entrance was just possible through a hygiene barrier where showering and change of clothes were required. One new stable for gilts was built and all previously housed pigs were slaughtered. 10 % of the new animals and their environment were sampled and tested for ESBL-producing *E. coli* and found to be negative. At the introduction of the pigs into the new stable they were given doxycycline orally as a prophylactic measure. Samples of environment, pigs, and air were taken two days before the old pigs left, two days after the entry of the new pigs, 30 and 60 days and 1 year after the introduction of the new batch of pigs. The samples taken 9 months before the decontamination showed a prevalence of 35 % of the pigs being colonized and shedding ESBL-producing *E. coli* in the faecal samples. The results of the second sampling at 1-2 days prior to the decontamination showed a decreased prevalence of 3.2 %. Regarding the environmental samples in 50 % (2/4) of the manure samples ESBL-producing *E. coli* have been found.

In none of the follow up investigations after the decontamination ESBL-producing *E. coli* could be detected. Even after one year the samples of the pigs and their environment did not show any ESBL-producing *E. coli*. One year later the farmer reported that he still sees long term benefits from the measures like decreased use of antibiotics due to healthier pigs

(Schmithausen et al. 2015a, Schmithausen et al. 2016). As a conclusion the radical decontamination and restructuring of the farm has been successful to eliminate ESBL-producing *E. coli* to a point of undetectability. It needs to be considered that these measures were aggressive and cost intensive. It would be interesting if less drastic/radical measures are able to eradicate ESBL-producing *E. coli*.

3.6.2.2. Bacteriophages

Another way to control ESBL-producing *E. coli* is by not just trying to stop the spreading but more so to reduce them. One new approach is to use bacteriophages. Bacteriophages are viruses of bacteria and similar to animal viruses they infect the bacterial cell and kill it (Duckworth and Gulig 2002). The idea here is to reduce the amount of resistant bacteria in healthy livestock animals rather than to cure infected animals that are sick. Skaradzińska and coworkers isolated phages from Polish pig farms to find out more about their effects on ESBL-producing *E. coli*. Since phages that are used individually, have a high variance in their effectiveness against *E. coli* they also tested how different bacteriophages would work together. Overall, 17 bacteriophages were tested on 104 ESBL/AmpC producing *E. coli*, which were previously isolated from farms in Germany. While a lot of the isolates showed a reduction in growth due to some of the used phages, 17.3 % did not respond to any of the tested phages. The bacteriophages lysed between 1.9 % to 57.7 % of the *E. coli* strains. Using a phage cocktail of three phages to cover a wider spectrum they showed that 80.8 % of the tested *E. coli* strains were lysed. This leads to the conclusion that phages have a potential to be used against ESBL-producing *E. coli*. Interestingly not all phages lyse the same strains and also not with the same efficacy. Therefore, it seems to be essential which phages or cocktails of phages are used against which bacterial strain. Nevertheless, the bacteriophages seem to be easily found and isolated from surrounding of the animals. Further studies are needed to answer the question if the bacteriophages should be sourced out of a different location or of the same environment as the bacteria strains (Skaradzińska et al. 2017).

3.6.2.3. Low Zinc diets

Zinc plays a big role in the physiological functions and is a cofactor for enzymes (Coleman 1992). Nevertheless, in the EU zinc in feedingstuff is just allowed up to 150mg/day or 150mg/kg

feedingstuff (Regulation (EC) No 1334/2003). Zinc supplements as veterinary products have been allowed to be used in higher amounts up to 2500mg zinc oxide/kg diet if only used for 14 days until July 2022. In 2022, Article 35 of Directive 2001/82/EC came into effect and prohibits the use of zinc oxide in amounts higher than 150 ppm in the EU (Directive 2001/82/EC). As previously described, there is evidence on the effects of a high zinc diet in piglets on resistance in *E. coli*. Ciesinski and coworkers described that piglets that got fed the low dose zinc diet showed significantly shorter persistence of resistant *E. coli* in the ileum than the group that got higher doses of zinc (Ciesinski et al. 2018). The research of Peng and coworkers is in line with these findings and also shows higher levels of ESBL-producing *E. coli* in naturally occurring *E. coli* when exposed to higher zinc concentrations (Peng et al. 2020).

As a result of different studies, it can be concluded that although a high zinc diet for piglets helps to prevent postweaning diarrhea it also promotes multiresistance, including resistance to ESBL-producing *E. coli*. This is in line with the EU regulation of a maximum zinc content of 150mg/kg in feedingstuff (Regulation (EC) No 1334/2003). High contents of zinc in pig feed are no alternative to in-feed antimicrobials. It should be preferred to use zinc in low doses from 80-150 mg/kg feedingstuff (Regulation (EC) No 1334/2003) to promote normal health but to prevent resistance to antimicrobials (Ciesinski et al. 2018).

3.6.2.4. No or little use of Cephalosporins

Several studies showed the impact of cephalosporin use on resistance to this antimicrobial class. Hammerum and coworkers showed a significantly higher prevalence of 79 % ESBL-producing *E. coli* in farms that used third- or fourth-generation cephalosporins compared to a prevalence of 20 % at farms that did avoid these antibiotics (Hammerum et al. 2014). The use of ceftiofur (third generation cephalosporin) has been shown to increase the number of ceftiofur resistant *E. coli* even after just one application (Beyer 2014).

In the study of Dohmen and coworkers, while not all antibiotics had a significant impact on the occurrence of ESBL-producing *E. coli*, cephalosporins were shown to always increase ESBL-producing *E. coli* significantly (Dohmen et al. 2017a). The work of Herrero and coworkers validates this hypothesis as they showed that samples treated with first and third-generation cephalosporins had a higher proportion of strains with multidrug resistance than untreated ones. They also showed that selective pressure was lower for cephalosporins compared to

ampicillin, but multiresistant strains were more frequently detected (Herrero-Fresno and Olsen 2020).

In conclusion antibiotics exert selective pressure on *E. coli*. Some antibiotics seem to have more impact on the development/occurrence of ESBL-producing *E. coli* than others. Evidence shows that cephalosporins promote ESBL-producing *E. coli* and should be avoided as much as possible in animal production from a resistance point of view.

4. Discussion

The aim of this literature review was to describe the prevalence of ESBL-producing *E. coli* in pigs and humans in contact with pigs. Secondly, we intended to identify the current knowledge on factors influencing the emergence and transmission of ESBL-producing *E. coli*. Furthermore, we tried to find measures that were evidently effective against ESBL-producing *E. coli*. This review showed that there are many factors influencing the prevalence of ESBL-producing *E. coli*. Internal and external biosecurity play a vital role in preventing the introduction and transmission of resistant bacteria. The results of this research showed that the prevalence of ESBL-producing *E. coli* in pigs varies heavily depending on the samples, the sample group, the country, and the farm management (especially the antibiotic regime/use). Some of the same variants apply to the human sample, but there is also some evidence that humans with direct contact to animals have a higher prevalence of ESBL-producing *E. coli*.

External biosecurity tries to control introduction routes of resistant bacteria into farms. The current science shows there are many ways that are proven to be a route for introduction of ESBL-producing *E. coli* including people entering the farm, vehicles, wildlife and even water and air. Some of these entry ways can be prevented through biosecurity like hygiene locks, pest control, fences to prevent wildlife contact and a water supply free of resistant bacteria. Most of these interventions have not been tested on farms having the same conditions but have just been investigated through analyses of data from existing farms.

On the other hand, internal biosecurity is essential to control the spread of bacteria within a farm. Farms with less intensive production systems have been shown to have lower prevalence of ESBL-producing *E. coli*. This phenomenon probably correlates with the animal density and the density of pig farms in production regions tend to have the same effect on the prevalence. Some structural measures seem to have more effect on ESBL-producing *E. coli* than others. Manure management, sick pens and ventilation play a role, but it is unclear which system is the most beneficial and these measures might also be different for individual farms. Hygiene including cleaning and disinfection might be one of the most important biosecurity measures. One measure that correlated with lower prevalence of ESBL-producing *E. coli* in several studies is pest control (preferably from professionals). Nevertheless, there are no consistent results in the current literature on other beneficial measures.

Antimicrobial usage and application route plays one of the biggest roles in the emergence of resistant bacteria. There is evidence that the use of cephalosporins promotes the selection of

ESBL-producing *E. coli*. The formerly promoted alternative zinc oxide shows similar effects on ESBL-producing *E. coli*. Individual treatment tends to have less effects on the resistance compared to group treatment. It has been shown that when group treatment is discontinued on a farm it increases the proportion of susceptible *E. coli* against most antibiotics. One concern is that oral antibiotics get eaten by animals in different doses. Underdosing is a mechanism that promotes resistance.

If ESBL-producing *E. coli* are present on a pig farm the most effective measure seems to be a radical decontamination of a farm combined with more intense biosecurity measures. This study of Schmithausen has been conducted on just one farm (without a control group on a second farm) but the measures included new livestock which was compared to the previous group.

Bacteriophages, especially bacteriophage cocktails are a promising intervention but have yet to be tested on pig farms.

4.1. Practicability of measures

The literature shows different approaches on the implementation of measures. It needs to be considered that some are more easily realized than others. Radical changes might be expensive and time-consuming. A radical decontamination as described by Schmithausen and coworkers is probably not achievable for most farms. Although this intervention was able to eradicate ESBL-producing *E. coli* it also required removal (slaughter) of all present pigs and the purchase of completely new animals which were free of the bug. It would be interesting to see if a decontamination could be achieved with less intensive measures. All structural measures that include building like flooring, manure system and hygiene locks are more cost intensive and should ideally be considered in the planning process of a farm. Management factors like cleaning regimes and antibiotic usage are easier to change but there might be difficulties in the implementation and occurring problems.

The reduction of the overall use of antibiotics is regimented by several governments. There were a few additional measures like the voluntary ban of cephalosporins in Denmark that resulted in a decrease of ESBL-producing *E. coli*. These measures by organizations and governments need to be well planned and based on evidence.

4.2. Quality (and heterogeneity of studies)

The quality of the studies differed greatly, both in the study plan and in presentation of the results. The main differences were in the size and time span of the conducted studies. While some were longitudinal studies with samples sizes of 16 pig fattening farms (Salviati et al. 2014) others described experiments with just 12 pigs as sample size (Beyer 2014). It should be considered that experiments under laboratory conditions might have different outcomes in stable conditions.

Another inconsistency of the comparison of farms was that risk factors sometimes seemed to be considered separately from the overall picture. The result “pest control by professionals” for example had a positive influence on the rate of ESBL-producing *E. coli* in the research of Dohmen and coworkers, but it should also be taken into account that farms that invest in professionals for this task probably have overall a higher biosecurity standard (Dohmen et al. 2017a). We also came across some overinterpretations like suggestions that ESBL-producing *E. coli* infections of humans could be airborne, even though the humans had direct contact with the animals (Dohmen et al. 2017b).

Some studies lacked precise descriptions and definitions. For example, we were not able to find out what exactly sayings like “separation/changing boots” or “no manure system” (Ketkhao et al. 2021) meant. The main focus of the Indian study of Mandakini was to find differences between “organized” and “unorganized” farms. We were surprised that no definition for these two terms were provided. Furthermore, we came across some misspellings that changed the meaning of the words. For example, one study (Gay et al. 2018) had “no lightning in the building” as a risk factor. We assume that the original meaning was “no lighting in the building”.

4.3. Discrepancies between studies

During this research there were a lot of discrepancies regarding the transmission of ESBL-producing *E. coli* from livestock to humans. Some studies suggested that the transmission is very likely because the same genes mediating the AMR were found in humans and livestock and that humans in contact with pigs (e.g. pig farmers) have higher rates of colonization with ESBL-producing *E. coli* than the average population. However, other studies had the conclusion that since no direct transmission of ESBL-producing *E. coli* from livestock could be

proved it is unlikely. Nguyen proposed that these varying outcomes might result from low granularity of the typing methods (Nguyen et al. 2021).

Although most studies point towards antimicrobial use as a risk factor for AMR, there are discrepancies between some studies. While most studies found an increase in AMR post antibiotic treatment, Poulin-Laprade and coworkers found the husbandry system with the least antibiotic use to be the one with the most ESBL phenotypes. It should be noted that there was no information about the previous use of antimicrobials, which could have influenced the occurrence of AMR. Another factor could be that the hygiene standard or health status of the pigs on the farms where no antibiotics were used was worse.

It seems that it is unclear if the use of sick pens is beneficial to control ESBL-producing *E. coli* or might even pose a risk of increased transmission. One explanation for the different results in the current research might be that the sick pens have different designs. Another big factor could be the way the pigs are transported to the separation pens. It would be beneficial if more detailed information regarding the sick pen is collected to find out which kind of sick pen is the most beneficial for the reduction of ESBL-producing *E. coli*.

It needs to be considered that most farms voluntarily participate in these studies. It could be problematic that the farmers that have less problems as regards antimicrobial resistance are the ones participating while the ones having more problems or high antimicrobial use fear conflicts and therefore deny access to their farms. This raises the question if there is a selection bias in many of the studies.

4.4. Data gaps

An assessment on transmission routes of β -lactamase producing *E. coli* in 2016 found that effects of climatic events like flooding need yet to be investigated. Horigan and coworkers identified farm factors like animal housing and “birthing pens” as data gaps. According to their study also bedding choices need more research to find out in which ways they act as reservoirs for ESBL-producing *E. coli* (Horigan et al. 2016). In this research we generally still found the same data gaps, but we discovered some research on the influence of flooring on ESBL-producing *E. coli*, which Horigan and coworkers had in their list of unresearched topics.

With a high probability there are other factors that could influence the occurrence and spreading of ESBL-producing *E. coli*. Friese and coworkers which found ESBL-producing *E.*

coli in the vicinity of farms including fertilized fields as described above, mentioned a few topics that might be of interest and need to be researched: “*Factors like temperature, rain, ultraviolet radiation, structure of the soil, concentration of heavy metals or the presence of other microorganisms could influence the viability of these pathogens.*” (Friese et al. 2013). We did not find any evidence in our literature research on these topics. These factors should be considered for further research in the field of ESBL-producing *E. coli* in pig farms.

There is some evidence on feeding strategies to reduce pathogen *E. coli* including specific information on ETEC. The interventions include pre- and probiotics and different kinds of starches and sugars (Tran et al. 2018). At this point we did not find research on the influence of these strategies on ESBL-producing *E. coli*. Some methods seem promising but further research is needed.

Some topics that we missed in the fields and suggest for further research are

- vaccines and their influences on antimicrobial use and resistant *E. coli*,
- influence of breed and origin of piglets on the prevalence of ESBL-producing *E. coli*,
- influence of occupancy density of pig stables on the prevalence of ESBL-producing *E. coli*,
- organic acids in livestock and their influence on ESBL-producing *E. coli*.

5. Summary

Escherichia coli (*E. coli*) is a common bacterial species in the intestines of humans and animals. Some kinds of *E. coli* can act as pathogens and most of them are considered zoonotic. Antimicrobial resistance has increased in the last years and is an important topic in the concept of One Health. Extended-spectrum β -lactamases (ESBL) producing *E. coli* are bacteria which are able to limit the effect of β -lactam antibiotics. Restricting the options of antibiotics, ESBL producing *E. coli* are furthermore able to limit the effect of the cephalosporins of the 3rd and 4th generation which are considered critically important antimicrobials by the WHO.

In this literature review the data bases Scopus, PubMed and Google Scholar were scanned for research on ESBL producing *E. coli* regarding potential risk factors and measures to reduce them in pig farms. We were able to find that the prevalence in pigs differs from 1.2 % to 85 % while the prevalence in humans was described from 6 % up to 86 % in the current literature. Although evidence is still inconclusive it has been shown that a lack of external biosecurity measures like no hygiene lock at the entrance, visitors and pets on the farm correlate with a higher prevalence of ESBL producing *E. coli* in pigs. Internal biosecurity measures including all-in-all-out systems and pest control by specialists correlate with lower prevalence of ESBL producing *E. coli* in pigs. One of the most important factors influencing resistance is antibiotic usage. This review revealed that the use of cephalosporins and group treatments seem to have a highly negative impact on the occurrence of ESBL producing *E. coli*.

Measures that could be implemented on pig farms to reduce the prevalence of ESBL producing *E. coli* are a radical decontamination, bacteriophages and low zinc diets.

6. Zusammenfassung

Escherichia coli (*E. coli*) gehören zu den häufigsten Bakterien der menschlichen und tierischen Darmflora. Sie können als Kommensalen aber auch als Pathogene agieren und haben häufig ein zoonotisches Potential. Antimikrobielle Resistenz ist ein Thema mit wachsender Bedeutung und spielt eine wichtige Rolle im Konzept „One Health“. Extended-Spektrum β -Laktamasen (ESBL) produzierende *E. coli* können β -Laktam-Antibiotika inaktivieren. Durch ihr erweitertes Wirkspektrum können sie unter anderem auch Cephalosporine der 3. und 4. Generation (von der WHO als kritische Antibiotika eingestuft) in der Wirksamkeit behindern und stellen damit eine große Herausforderung für die Medizin dar.

In diesem Literatur-Review wurden die Datenbanken Scopus, Pubmed and Google Scholar genutzt und nach Forschungsergebnissen zu ESBL produzierenden *E. coli* in Schweinebeständen im Bereich Risikofaktoren und Maßnahmen zur Eindämmung durchsucht. Die Prävalenz von ESBL produzierenden *E. coli* variiert in der Literatur von 1,2 % bis 85 % bei Schweinen und von 6 % bis 86 % bei Menschen.

Fehlende Maßnahmen zur Verhinderung des Eintrags (externe Biosicherheit), wie beispielsweise keine Hygieneschleuse, Besucher und Haustiere in den Farmen korrelieren mit einer erhöhten Prävalenz in Schweinen. Als Maßnahmen der internen Biosicherheit haben ein Rein-Raus-Verfahren sowie Ungezieferbekämpfung durch Spezialisten eine negative Korrelation mit der Prävalenz von ESBL produzierenden *E. coli* in Schweinen gezeigt. Neben dem Antibiotikaeinsatz per se scheinen vor allem Cephalosporine sowie die Entscheidung zu einer Gruppenbehandlung eine große Rolle in der Steigerung der Prävalenz von ESBL produzierenden *E. coli* zu spielen.

Als Maßnahmen zur Reduktion der Prävalenz von ESBL produzierenden *E. coli* haben sich eine radikale Dekontamination, Einsatz von Bakteriophagen sowie Fütterung mit einer geringen Zink-Menge als wirkungsvoll herausgestellt.

Bisher gibt es wenige eindeutige Ergebnisse und es besteht ein großer Bedarf an weiterer Forschung.

7. List of Abbreviations

AIAO	All-in-all-out
AmpC	Ampicillinase C
AMR	Antimicrobial resistance
APR	Apramycin
BPC	Breakpoint concentration
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
CREC	Cefotaxime-resistant <i>E. coli</i>
DDDA/Y	Defined Daily Dosages per Animal Year
DPBS	Dulbecco's Phosphate Buffered Saline
<i>E. coli</i>	<i>Escherichia coli</i>
EFSA	European Food Safety Authority
EHEC	Enterohaemorrhagic <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ESBL	Extended-spectrum β -lactamases
ESC	Extended-spectrum cephalosporins
ESC-R-EC	Extended-spectrum cephalosporin-resistant <i>Escherichia coli</i>
EU	European Union
MALDI-ToF MS	Matrix assisted laser desorption ionization-time of flight mass spectrometry

MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NON SEL	Nonselective methods were used for growing <i>E. coli</i> .
PFGE	Pulsed-field gel electrophoresis
PKPD	Pharmacokinetic-pharmacodynamic
ppm	Parts per million
SEL	Selective methods were used for growing <i>E. coli</i> .
SXT	Trimethoprim-sulfamethoxazole

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Data taken from (Lucia et al. 2021)

9. References

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