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Review

Types and prevalence of extended–spectrum beta–lactamase producing *Enterobacteriaceae* in poultry

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Abstract

For several billion years, bacteria have developed mechanisms to resist antibacterial substances. In modern time, antibiotics are frequently used in veterinary and human medicine for prevention and treatment of diseases, globally still also for their growth promoting effects as feed additives. This complex situation has evolved in accelerating development and prevalence of multi-drug resistant bacteria in livestock and people. Extended-spectrum beta-lactamase (ESBL) producing bacteria are resistant to a wide range of ß-lactam antibiotics. They are currently considered as one of the main threats for the treatment of infections in humans and animals. In livestock and animal products, poultry and poultry products show the highest prevalence of ESBL-producers with CTX-M-1, TEM-52 and SHV-12 being the most common ESBL-types in poultry. *Escherichia coli* and *Salmonella* spp. are the bacteria in poultry, which carry ESBL-genes most frequently. ESBL-producing bacteria are present at every level of the poultry production pyramid and can be detected even in the meconium of newly hatched chicks. The environment close to poultry barns shows high prevalence rates of these bacteria and contributes to an ongoing infection pressure with further ESBL-types. Probiotics have been shown to successfully reduce ESBL-producers in chicken, as well as ESBL-gene transfer. Other feed additives, such as zinc and copper, increase the prevalence of ESBL-producing bacteria when fed to animals. To our best knowledge, this is the first publication presenting a comparative overview of the prevalence of ESBL-types using data from different countries. To reduce the hazard for public health from poultry carrying high numbers of ESBL-producers, preventive measurements must include the surrounding environment and avoidance of antibiotic usage at all levels of the production pyramid. The first results, of the research on the impact of feed additives on the spread of ESBL-genes, indicate the diet as a further, possible magnitude of influence.

Keywords: broilers, ESBL, antibiotic resistance, feed additives.

Introduction

Usage of antibiotic growth promotors has been a common practice in European poultry farming in order to increase the performance until the phasing out in the year 2006. The raising awareness on the hazard subsequent to antibiotic feed additives in animal farming has led to the ban of such in European poultry production (Regulation (EC) No 1831/2003 chapter II article 11). Nevertheless, poultry meat still carries the highest contamination with ESBL-producing bacteria compared with other meat sources (Geser *et al.*, 2012; Carmo *et al.*, 2014; De Jong *et al.*, 2014). Cephalosporins belong to the ß-lactam antibiotics, which can be hydrolyzed by extended-spectrum ß-lactamases and thereby exert a selective pressure on ESBL-producing bacteria (Paterson and Bonomo, 2005). Despite that several countries, such as Sweden and Belgium, do not use cephalosporins for poultry, a high prevalence of

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ESBL-producing bacteria remains (Smet *et al.*, 2008; SVARM 2011, 2012). This suggests that there are additional sources for the contamination with ESBL-producing bacteria in poultry farming (Hiroi *et al.*, 2012a).

Worldwide, chicken was the second most common meat source (35.2%) in 2012, behind pork (36.3%) and followed by beef (22.2%) (http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html). Poultry is the meat source with the highest percentage rise between 1990 and 2012 (104.2%) according to the Food and Agriculture Organization of the United Nations (FAO) (http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html). ESBL-producing bacteria are able to transmit from poultry and poultry products to humans (Bertrand *et al.*, 2006; Leverstein-Van Hall *et al.*, 2011). The transmission from poultry to humans as food borne diseases or through the environment leads to a major hazard for public health (Apata, 2009; Davies and Davies, 2010). Infections with multi-drug resistant bacteria, such as ESBL-producing bacteria, are difficult to treat and cause high morbidity and increased mortality in humans (Davies and Davies, 2010). Trivially, the accountable bacteria are therefore often referred to as 'superbugs'. Examples for such bacterial species are *Escherichia coli, Salmonella enterica* and *Klebsiella pneumoniae*, all common bacteria in the gastrointestinal tract of poultry (Rehman *et al.*, 2007; Apata, 2009; Davies and Davies, 2010). Third-generation cephalosporins are frequently used to treat humans with difficult-to-cure infections caused by *Enterobacteriaceae* (Koga *et al.*, 2015). Currently, a rising occurrence of bacteria resistant to third-generation cephalosporins causes concern. In 2014, the national prevalence in Europe reached from 3.3 to 40.4% leading to an increase of the mean percentage from 9.6% (2011) to 12.0% (2014) in the EU/EEA (European Economic Area) (Ears-Net, 2015). A major part of these bacteria produces ESBL. The European Antimicrobial Resistance Surveillance System (EARSS) reported a national prevalence in ESBL-producing bacteria in the EU/EEA between 73.6 to 100% in 2013 and 71.1 to 100% in 2014 (Ears-Net, 2014, 2015). In the EU, the growing awareness on multi resistant bacteria and the impact of co- and cross selection has led to a ban on antibiotic feed additives (Regulation (EC) No 1831/2003 chapter II article 11) and new adjustments on recommended mineral contents in feed (EFSA: https://www.efsa.europa.eu/en/press/news/160809a.). According to the World Health organization, ESBL-producing *Enterobacteriaceae* belong to the most urgent health issues (World Health Organization, 2014). It is therefore crucial to identify and reduce the ESBL load in poultry farming.

To our best knowledge, this is the first review comparing types and prevalence of ESBLs common in poultry. This information is vital hence to the possible transmission of ESBL genes from commensals to pathogens and from poultry to humans.

Classification of ESBL genes

Due to the transmission of plasmids with genes encoding for ESBLs between different species (Apata, 2009) and the absence of correlation between ESBL-type and ESBL-producing bacteria, a categorization of ESBL by bacterial species is not considered useful. Hence, these bacteria are classified based on the amino acid sequences of the ESBL. They can be functionally classified into the group of Ambler's class A (TEM, SHV, CTX-M, PER, VEB, GES, TLA, BES) and D (OXA) lactamases according to Ambler *et al.* (1991) (Bush *et al.*, 1995; Gniadkowski, 2001). Worldwide, TEM (named after the patient Temoniera), SHV (sulphydryl reagent variable), and CTX-M (hydrolyses cefotaxime) are the most frequently detected ESBL-types (Canton *et al.*, 2008). The genes encoding for these enzymes are termed bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$. These also represent the major ESBL-types observed in poultry and poultry products. Within the groups, there are a number of subtypes.

ESBL-producing bacteria have the ability to interchange ESBL-genes within and across species (Apata, 2009). The Genes encoding for ESBLs are located on mobile elements (plasmids, integrons or transposons), but can also be found on the bacterial chromosome. An active transposition from plasmid to genome was suggested by Shahada *et al.* (2013). The ESBL-genes spread vertically through cell division or by gene transfer within one species, but also horizontally to other species and genera by conjugation (Händel *et al.*, 2015; Yamaichi *et al.*, 2015; Porse *et al.*, 2016). In poultry, ESBL-genes are found on a variety of plasmid types where IncI1 and IncFIB are examples for frequently detected types (Supplementary data). Beside ESBL genes, these plasmids often carry genes that encode resistance to other antibiotics or heavy metals and are therefore often co-selected, especially when located close to each other (Silver and Phung, 1996; Meunier *et al.*, 2006; Liu *et al.*, 2011; Seiler and Berendonk, 2012; Borjesson *et al.*, 2013)

Recent development of ESBL in poultry

Although antimicrobial resistance is an ancient phenomenon, prevalence of ESBL-producing bacteria in poultry became significantly higher after the usage of ß-lactam antibiotics (Dierikx *et al.*, 2013b). Besides mutations in genes encoding for ESBL, selective pressure influences other genes, promoters and the quantity of ESBL-genes enhancing the resistance against antibiotics. A change of porin proteins through mutation can e.g. alter the outer membrane permeability for antibiotics. The emergence of resistances against other groups of antibiotics can lead to co-selection and stronger promoters can increase the expression of ESBL-genes (Gniadkowski, 2001, 2008).

The exposure of bacteria to ß-lactam antibiotics in poultry farming through feed additives and clinical or preventive treatments leads to the elimination of sensitive strains but spares resistant bacteria. Merely these 'persisters' are able to multiply in the presence of ß-lactam antibiotics. They can increase tremendously due to selective pressures leading to an increase in resistance against ß-lactams (Apata, 2009; Poole, 2012). Furthermore, ESBL-producing bacteria may serve as a gene reservoir for other strains and species (Apata, 2009). Another possible way for bacteria to obtain ESBL-genes is through

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interactions with environmental bacteria, which can hold both ancient or recently developed antibiotic resistance (Galan *et al.*, 2013).

Selective pressure, due to the usage of antibiotics, may enhance the emergence and rise of antibiotic resistance. In the case of ESBL-producing bacteria, ß-lactam antibiotics can be at the root of the resistance (Dierikx *et al.*, 2013b). Declining prevalence of ESBL-producing bacteria in broiler meat and intestinal content were observed subsequent to the ban of antibiotic growth promotors in Denmark, Sweden and the Netherlands (DANMAP, 2015; Swedress-Svarm, 2015; Veldman *et al.*, 2016). Correspondingly, the occurrence of bacteria resistant to cefotaxime[1] in fecal samples from broilers reduced significantly after a national ban on the usage of ceftiofur in Dutch hatcheries in 2010 from about 18% (2010) to under 10% (2011) (Koene *et al.*, 2012). These data show an immediate effect from antibiotic reduction measurements on the prevalence of antibiotic resistance. Nevertheless, there are several reports on an occurrence of ESBL-producing bacteria in broilers and their products superior to the incidence in other livestock. The high prevalence of ESBL in broilers is remarkable since many countries, such as Denmark, Belgium and Sweden, have banned the usage of cephalosporins for poultry but not for other livestock (Smet *et al.*, 2008; Bengtsson *et al.*, 2012; Kameyama *et al.*, 2013). This must lead to the assumption that other factors besides the usage of ß-lactam antibiotics affect the spread and prevalence of ESBL- producing bacteria in poultry.

As mentioned, antibiotic resistance against different types of antibiotics can be co-selected (Meunier *et al.*, 2006; Liu *et al.*, 2011). Examples for co-resistance against ß-lactams and heavy metals are silver and CTX-M-15 and CTX-M-14, mercury and SHV and TEM or mercury and ampicillin among others. Copper and zinc may promote multi resistant bacteria (Sutterlin *et al.*, 2014; Yazdankhah *et al.*, 2014; Vahjen *et al.*, 2015). Co-resistance also exists among different antibiotic classes, i.e. ESBL-producers may hold co-resistance to fluoroquinolone, tetracycline and/or trimethoprim (Shashwati *et al.*, 2014; Tacao *et al.*, 2014; Bajaj *et al.*, 2016). A high percentage (98%) of the ESBL-producing strains obtained from chicken carcasses in Brazil also expressed resistance to tetracycline (Koga *et al.*, 2015). ESBL-producing *E. coli* occurred with a high prevalence (34% in 2010 and 54% in 2011) in Swedish broiler flocks. Genes encoding for CTX-M were found on an IncI1 plasmid together with resistance against tetracycline and sulfamethoxazole, suggesting co-selection as a source for the high prevalence (Borjesson *et al.*, 2013). Nevertheless, low usage of not only cephalosporins but also other antibiotics (0.19% of all flocks) (Bengtsson *et al.*, 2012) must lead to the assumption that the resistance against ß-lactam antibiotics growing in poultry is also due to reasons other than antibiotic usage (Borjesson *et al.*, 2013). Especially when compared with the usage of antibiotics in other livestock, which show significantly lower contamination rates with ESBL-producing bacteria despite higher consumption of antibiotics (Bengtsson *et al.*, 2012; Borjesson *et al.*, 2013). The prevalence of ESBL-producing *E. coli* was compared in three flocks of broilers, one treated with antibiotics (not cephalosporins), one without and one fed antibiotics and kept in laboratories, which never housed poultry before. Surprisingly, the first two flocks showed a high occurrence of ESBL, while the flocks kept in the laboratories showed much lower contamination with ESBL-producing bacteria (Hiroi *et al.*, 2012a). This implies that the environment plays a major role, even more important than antibiotic co-selection. Correspondingly, high contamination levels with ESBL-producers were observed in the environment close to barns housing poultry (Blaak *et al.*, 2015). The bacteria may spread to the environment by waste products from animal production (Apata, 2009). The high prevalence of ESBL-producers in the environment may also explain the high prevalence of ESBL-producing bacteria in organic broiler flocks (Stuart *et al.*, 2012).

Antibiotic and heavy metal resistance are often observed simultaneously. Bacteria, which frequently carry plasmids with both genes encoding for biocide/metal resistance and antibiotic resistance genes, are *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* spp., *Enterococcus* spp. and *Escherichia* spp. (Pal *et al.*, 2015). The prevalence of co-resistance plasmids is significantly higher in humans and domestic animals than in other environments such as wild animals, soil, plants or food (Pal *et al.*, 2015). ESBL-producing bacteria from a hospital environment showed co-resistance to cadmium, copper, mercury and lead, but not zinc (Touati *et al.*, 2010). Silver resistance was associated with CTX-M-15 and CTX-M-14 in humans but not in birds, while mercury resistance genes were found together with SHV and TEM genes in both human and avian samples (Sutterlin *et al.*, 2014). Hence to these common co- and cross-selection of antibiotic resistance and heavy metal resistance, it is to no surprise that mineral feed can influence the gut resistome (the gathered bacterial genetic pool of antibiotic resistance, regardless if pathogenic or not (Wright, 2007)). High levels of zinc and copper supplementation may promote multi resistant bacteria (resistant against three or more antibiotics) in animals and animal excretions (Yazdankhah *et al.*, 2014; Vahjen *et al.*, 2015). Zinc supplementation was associated with evaluated resistance against antibiotics such as ampicillin, piperacillin, doxycycline, penicillin, tetracycline and sulfonamide/trimethoprim in pigs. It also enhanced the prevalence of ESBL-producing *E. coli* and methicillin resistant *Staphylococcus aureus*, both causing difficult-to-treat infections in humans and animals (Aarestrup *et al.*, 2010; Holzel *et al.*, 2012; Bednorz *et al.*, 2013; Vahjen *et al.*, 2015). Copper supplementation led to increased resistance against macrolides, glycopeptides, ampicillin, amoxicillin/clavulanic acid and piperacillin (Hasman and Aarestrup, 2002; Holzel *et al.*, 2012). Feed supplements, which tend to reduce antibiotic resistance, are mercury (also reducing the prevalence of multi resistant bacteria) and, with less impact, lead. Co-resistance to cadmium and ß-lactams was detected irregularly in pigs (Hustavova *et al.*, 1994; Holzel *et al.*, 2012). Nickel and chrome had no impact on observed resistance in pigs' excretions (Holzel *et al.*, 2012). Besides from co-selection, an increased uptake of plasmids, due to mineral

[1]Cefotaxime is a third-generation cephalosporin commonly indicating ESBL-resistance in bacteria.

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Table 1. Recent prevalence of ESBL in Enterobacteriaceae in poultry (2006–2011)

Prevalence (%)	Country of sample collection	Reference
93.0[1]	Denmark	Agerso *et al.* (2014)
27.0[2]		Agerso *et al.* (2014)
3.3–8.6[3]		Agerso *et al.* (2014)
94.5[3]	Finland	Lyhs *et al.* (2012).
43.9–88.6[3]	Germany	Kola *et al.* (2012), Lyhs *et al.* (2012), Reich *et al.* (2013), Belmar Campos *et al.* (2014)
81.0–85.5[2]		Laube *et al.* (2013), Blaak *et al.* (2015)
65.0[4]		Blaak *et al.* (2015)
57.7[3]	Japan	Kawamura *et al.* (2014)
94.0[3]	Netherlands	Leverstein-Van Hall *et al.* (2011)
84.0[5]–100.0[6]		Stuart *et al.* (2012)
85.0[2]		Dierikx *et al.* (2013a)
15.0–44.0[7]		Dierikx *et al.* (2013b)
0.3–5.8[1]		Dierikx *et al.* (2013b)
79.7[2]	Spain	Blanc *et al.* (2006)

[1]Broiler parent flocks.
[2]Broiler flocks.
[3]Poultry meat.
[4]Laying hens.
[5]Organic poultry farming.
[6]Conventional poultry farming.
[7]Broiler grandparent flocks poultry.

interaction, may be the reason for co-resistance as a consequence of mineral feed supplementation (Bednorz *et al.*, 2013).

ESBL-producing bacteria and resistance types in broilers

The majority of ESBL producing bacteria in poultry are *E. coli* and *Salmonella* spp. (Table 1). ESBL-producing *E. coli* belong to the phylogenic groups A, B1, B2, D and E (Table 2). While A and B1 are considered part of the commensal intestinal community, B2 and D are linked to pathogenic activity (Herzer *et al.*, 1990; Clermont *et al.*, 2000). The most frequent detected ESBL type in poultry is CTX-M (De Jong *et al.*, 2014; Tschudin-Sutter *et al.*, 2014; Valentin *et al.*, 2014) (Table 5). TEM and SHV are predominant in subclinical infections in poultry while TEM and CTX-M dominate samples taken from poultry with disease-associated symptoms (Olsen *et al.*, 2014).

ESBL producing *E. coli* were detected in the meconium of 1-day-old chickens showing a tendency of preservation of genotypes throughout the poultry production pyramid. This implies that ESBL genes are transmitted clonally and vertically throughout the entire poultry production pyramid even without antimicrobial selection pressure (Koene *et al.*, 2009; Dierikx *et al.*, 2013b; Laube *et al.*, 2013; Agerso *et al.*, 2014; Olsen *et al.*, 2014; Zurfluh *et al.*, 2014). In the Netherlands, ESBL-producing bacteria are suspected to be introduced to the poultry producing system through imported 1-day-old grandparent chicks. This could be a further explanation for the high prevalence of ESBL-producing bacteria in organic broiler flocks (Stuart *et al.*, 2012). The prevalence of ESBL-producing bacteria is hence much higher in 1-day-old grandparent chicks than in 1-day-old parent chicks, who in return show higher contamination than 1-day-old broiler chicks (Koene *et al.*, 2009; Dierikx *et al.*, 2013b). Combined with the fact that CTX-M producing *Enterobacteriaceae* cause food-borne diseases, which are difficult to treat, a hazard to human health arises from poultry production even without the usage of antimicrobial feed additives. Evidence that intraspecies transmission of ESBL encoding genes takes place in *Salmonella enterica* and *E. coli*, was found (Shahada *et al.*, 2013). Besides from the vertical transmission, the contaminated environment seems to be crucial for the infection of poultry. While bla_{CMY-2} was the only ß-lactamase gene found at the top of the poultry production pyramid, other types were found in older grandparent chickens and in 1-day old parent chicks and broilers. The same enzyme types were isolated in samples taken from the environment, suggesting this to be the source of the infection with the additional ESBL-types (Dierikx *et al.*, 2013b).

ESBL-producing bacteria and resistance types in poultry products and their transmission to humans

Poultry meat shows the highest contamination of ESBL-producing bacteria compared with other meat sources (Friese *et al.*, 2013). Different research groups independently found a high prevalence of ESBL-producing bacteria in products from poultry while meat origin from other livestock showed significantly lower contamination. Contamination of broiler feces and objects in close contact with broiler meat reached up to 100% (Table 1). In a Danish study carried out from 2009 to 2011, poultry meat carried the highest contamination of the examined meat sources (human exposure to ESBL-producing bacteria: 83.8% from broiler meat, 12.5% from pork and

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Table 2. Recent prevalence of *E. coli* phylogenic groups in Enterobacteriaceae in poultry (2003–2013)

Phylogenic group	Prevalence (%)	Country of sample collection	Reference
A	31.5–34.0	Germany	Reich *et al.* (2013), Blaak *et al.* (2015)
	13.3–28	Netherlands	Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	37.7	Not specified	Lyhs *et al.* (2012)
	3.85–36.5	Spain	Cortes *et al.* (2010), Egea *et al.* (2012)
B1	20.2–42.0	Germany	Reich *et al.* (2013), Blaak *et al.* (2015)
	20–38	Netherlands	Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	28.8–38.6	Spain	Cortes *et al.* (2010), Egea *et al.* (2012)
B2	5.4–13.5	Germany	Reich *et al.* (2013), Blaak *et al.* (2015)
	2–8.9	Netherlands	Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	7.7	Not specified	Lyhs *et al.* (2012)
	0.0–7.0	Spain	Cortes *et al.* (2010), Egea *et al.* (2012)
D	17.7–34.8	Germany	Reich *et al.* (2013), Blaak *et al.* (2015)
	4.4–32.2	Netherlands	Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	50.7	Not specified	Lyhs *et al.* (2012)
	0–32.6	Spain	Cortes *et al.* (2010), Egea *et al.* (2012)
E	3	Netherlands	Kluytmans *et al.* (2013)

3.7% from beef) (Carmo *et al.*, 2014). Equally, poultry meat was found to be contaminated with ESBL-producing *Salmonella* spp. to a higher extent than pork meat (De Jong *et al.*, 2014). In Germany and Switzerland, feces were collected from healthy poultry, pigs and cattle. Again, samples from broilers showed the highest contamination rate (100% respectively, 63.4%) followed by cattle (60% respectively, 13.7%) and pigs (up to 56.3% respectively, 15.3%) by ESBL-producing *E. coli* (Geser *et al.*, 2012; Friese *et al.*, 2013). In Japan, broilers carry the highest contamination with ESBL-producing *E. coli* (broilers 60.0%, laying hens 5.9%, cattle 12.5% and pigs 3.0%) (Hiroi *et al.*, 2012b). In the Netherlands, ESBL-producing *E. coli* were detected in 79.8% of the examined chicken meat compared with 4.7% in beef and 1.8% in pork (Overdevest *et al.*, 2011). Within poultry, broilers are contaminated with ESBL-producing bacteria to a higher extent than laying hens (Hiroi *et al.*, 2012b; Blaak *et al.*, 2015; Evers *et al.*, 2016).

Interestingly, ESBL-producing bacteria show a high prevalence both in conventional and organic poultry farming and retail meat products (Stuart *et al.*, 2012) (Table 1). This is surprising considering the strict limitation of antibiotic usage in organic farming. However, when comparing chicken carcasses from free-ranged chicken with those of conventional raised poultry in different countries, the prevalence of extended-spectrum ß-lactamase producers depended on many factors where the production system is not decisive (Blaak *et al.*, 2015; Koga *et al.*, 2015; Mancabelli *et al.*, 2016). Important variables, such as animal housing, feed or the size of flocks of defined poultry production systems, differ between countries. This complicates the comparison of data on the prevalence of ESBL-producers in different production systems from studies carried out in different countries.

Because products from broilers have the highest ESBL contamination among all animal products, it is not surprising that, when it comes to the ESBL transmission from livestock products to people, poultry is considered as main source. The relationship between ESBL-producing *E. coli* isolates obtained from poultry and humans was closer than the one between ESBL-producing *E. coli* from pig and human origin (Cortes *et al.*, 2010). A high similarity was observed between plasmids encoding CTX-M-1 isolated from *E. coli* and *Salmonella* spp. strains of poultry origin. Conversely, they showed differences to CTX-M-1-encoding plasmids from other animal origins (Cloeckaert *et al.*, 2010). This led to the assumption that poultry might be the origin of plasmids encoding CTX-M-1.

The ability to transfer genes encoding for ESBL from an *E. coli* strain from poultry origin to an *E. coli* recipient with human origin has been frequently reported. The mechanism behind this is the transfer of plasmids by conjugation. Because these plasmids often carry additional genes encoding for other types of antibiotic resistance, they are transferred together with the resistance against ß-lactams (Meunier *et al.*, 2006; Liu *et al.*, 2011). *E. coli*-strains isolated from broiler feces may both proliferate and become part of the simulated human gastrointestinal tract. Simultaneously, plasmids encoding for ESBL can be transferred from the *E. coli* poultry strain to *E. coli* strains of human origin (Smet *et al.*, 2011). CTX-M-2 was identified in Belgium and France, samples containing this enzyme were chronologically obtained first from poultry flocks, then from poultry meat and finally from humans. This led to the assumption that poultry was the source of the infection in humans (Bertrand *et al.*, 2006). Comparing ESBL-genes, plasmids and strain genotypes in *E. coli* from poultry, chicken meat and human sources, a close relation between isolates from human and animal origin was observed (Leverstein-Van Hall *et al.*, 2011). This suggests that poultry might be a reservoir for ESBL producing bacteria (Girlich *et al.*, 2007). Beside food borne infection, flies may function as a possible vector for transmission of ESBL-producing *E. coli* from poultry to humans (Blaak *et al.*, 2014, 2015). A quantitative microbiological risk assessment investigated the exposure of humans to ESBL-producing *E. coli* originating from poultry at a worst-case scenario in 2013. Comparing chicken fillets and flies as possible sources for the transmission, the evaluation identified a higher public health risk due to ESBL-producing bacteria originating from chicken fillets than from flies (Evers *et al.*, 2016).

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Although ESBL-producing bacteria are classified as food-borne pathogens originating from livestock products, the prevalence of different ESBL types can differ between people and livestock. CTX-M-1 was found the most common ESBL type in livestock (pigs, cattle and poultry) in Germany, followed by a combination of CTX-M-1 and TEM (Valentin *et al.*, 2014). In the same study, $bla_{CTX\text{-}M\text{-}15}$ was the main gene found in samples from humans. In the Netherlands, CTX-M-1 and SHV-12 were the most common types of ESBL in bacteria from both humans and poultry (Huijbers *et al.*, 2014). Further studies found CTX-M and TEM-52 to be the predominant enzymes in ESBL-producing *E. coli* from both humans and poultry (Leverstein-Van Hall *et al.*, 2011; Overdevest *et al.*, 2011). In a Dutch study, samples from humans and retail chicken meat were compared for their ESBL-producing *E. coli*. A high similarity in mobile resistance elements, virulence genes and genomic backbones were detected (Kluytmans *et al.*, 2013).

Whether poultry meat is the reason for human infection with ESBL-producing bacteria or only serves as a reservoir for ESBL-producing bacteria is currently under discussion. In humans, ESBL-producing bacteria are often associated with urinary-tract-infections or the nosocomial bacterial community (Dierikx *et al.*, 2013a; Huijbers *et al.*, 2014; Valentin *et al.*, 2014). A review of the infection with ESBL-producing bacteria through products of livestock origin identified poultry as a major source (Lazarus *et al.*, 2015). ESBL-producing bacteria from poultry may reach the environment with waste products, manure and excretions. They may proceed to humans through surface water, vegetables and fruits as well as when handling the animals. Poultry products, especially when treated with poor hygiene, are suggested to be another major source of human infection with ESBL-producing bacteria (Apata, 2009).

Prevalence of ESBL types in poultry farms and products

Plasmids carrying genes encoding for ESBL often carry genes for more than one ESBL-type (Supplementary data). The prevalence of ESBL-producing bacteria in poultry and their products differs between countries, years and products, reaching from 3.3% in retail broiler meat in Denmark (2009) to 100% in conventional farmed flocks (2010) in the Netherlands. In 10 out of 14 cases, ESBL-producing bacteria were observed in more than 50% of the collected samples (Table 1). Furthermore, the prevalence of different ESBL types varies between countries, years and products (Tables 3–5). TEM-52 is the most frequently detected TEM type in poultry (Table 3). SHV-2 and SHV-12 are frequently detected in poultry. In studies comparing the occurrence of SHV-2 and SHV-12, SHV-12 positive samples are usually observed with a higher prevalence (Table 4). CTX-M is widely spread in poultry production. It occurs with high prevalence of up to 100% (UK) (Wu *et al.*, 2013). Many different CTX-M-types have been identified in samples from poultry. CTX-M-1 demonstrates to be the most important type of CTX-M enzymes in poultry production. However, a high variation can be observed between different countries (Table 5). While there is a high prevalence for CTX-M in many European countries, it is comparably low in Japan and China. European countries, with a high prevalence for detected CTX-M-1 enzymes from poultry samples, are the UK, the Netherlands and Germany. Spain belongs to the European countries with a low CTX-M-1 prevalence. Conversely, CTX-M-2 was observed with a high prevalence in Japan (51.2%) and a low prevalence in Europe (0–9%). CTX-M-15, being the predominant CTX-M type in humans (Valentin *et al.*, 2014), shows a relatively low prevalence in poultry products. The prevalence reaches from 0% in studies from Germany, the Netherlands and Spain to 17% in a study on poultry products from the UK (Table 5).

Suggested strategies to combat ESBL in chicken

By feeding a commercial competitive exclusion product comprising a defined mixture of commercial bacteria, consisting of *E. coli* strains with susceptibility against antibiotics and other microorganisms, the ESBL-producing *E. coli* could be reduced in the cecal content of broilers (Nuotio *et al.*, 2013). To the best of our knowledge, this is the only publication about the influence of probiotics on ESBL-producing bacteria in poultry. The inhibiting effect of probiotic *Bifidobacterium* spp. strains on the spread of ESBL-genes was demonstrated in an in-vitro experiment and confirmed in gnotobiotic mice. While *Bifidobacterium bifidum* and *Bifidobacterium pseudocatendatum* declined SHV-5 and CTX-M-15 gene transfer from a donor to a recipient by around 3 logs, *Bifidobacterium longum* failed to reduce the transconjugation frequency. Hence to the constant quantities of donors and recipients, the effect was suggested to result from metabolites, inhibiting the transfer of plasmids, rather than from an antibacterial effect of the probiotic bacteria (Moubareck *et al.*, 2007). Detection of ß-lactamases (not defined) in the feces of children treated with ceftriaxone reduced from 60% to 30–40% when treated with different mixtures of *Bifidobacterium* spp. and *Lactobacillus* spp. (*B. bifidicum*, *Lactobacillus acidophilus* and others). Other probiotics were less effective or even increased ß-lactamases (*Saccharomyces boulardii* and *Lactobacillus cusei* ssp. *rhamnosus* GG). Lactulose, a prebiotic, had no impact on the prevalence of ß-lactamases in the examined samples (Zoppi *et al.*, 2001). These results demonstrate the importance of detailed research, hence the protective capacities may differ within one species. Regardless of the urgent need of further comprehensive research on this topic, this suggests that probiotic or synbiotic feed additives may reduce antibiotic resistance in poultry production successfully. Research on the impact of feed additives, such as probiotics, prebiotics and organic acids, on the gastrointestinal bacterial community in poultry has been versatile, specific and directed (Van Immerseel *et al.*, 2006; Williams, 2010; Zalan *et al.*, 2010; Huyghebaert *et al.*, 2011; Alloui *et al.*, 2013). However, research on their impact on antibiotic resistant bacteria has been very sparse.

The mechanisms responsible for the antagonistic effect of probiotics towards pathogens are versatile and often strain-

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Table 3. Recent prevalence of TEM in Enterobacteriaceae in poultry (2006–2013)

Enzyme	Prevalence in (%)	Country	Reference
TEM	27.0	Germany	Reich *et al.* (2013)
	0.0–1.6	Spain	Cortes *et al.* (2010), Egea *et al.* (2012)
TEM-1	12.54	Germany	Laube *et al.* (2013)
	41.2	Belgium	Smet *et al.* (2008)
TEM-19	0.0	Netherlands	Kluytmans *et al.* (2013)
TEM-20	0.0[1]–3.0[2]	Netherlands	Stuart *et al.* (2012)
	1.0[3]–3.0[4]	Netherlands	Leverstein-Van Hall *et al.* (2011)
TEM-52	9.1–14.0	Netherlands	Overdevest *et al.* (2011), Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	20.0[2]–42.0[1]	Netherlands	Stuart *et al.* (2012)
	26.0[3]–29.0[4]	Netherlands	Leverstein-Van Hall *et al.* (2011)
	8.6–10.0	Germany, Switzerland	Geser *et al.* (2012), Kola *et al.* (2012)
	3.4–28.0	Germany	Laube *et al.* (2013), Belmar Campos *et al.* (2014), Blaak *et al.* (2015)
	2.33	Japan	Kawamura *et al.* (2014)
	3.1	Spain, Catalonia	Blanc *et al.* (2006)
	13.7–43.1	Belgium	Smet *et al.* (2008), De Jong *et al.* (2014)
TEM-106	2.0	Belgium	Smet *et al.* (2008)

[1]Organic farming.
[2]Conventional farming.
[3]Chicken meat.
[4]Poultry.

Table 4. Recent prevalence of SHV in Enterobacteriaceae in poultry (2003–2013)

Enzyme	Prevalence (%)	Country of sample collection	Reference
SHV	11.0	Denmark	Agerso *et al.* (2014)
	0.0–47.0	Germany	Reich *et al.* (2013), Wu *et al.* (2013), Belmar Campos *et al.* (2014)
	2.33	Japan	Kawamura *et al.* (2014)
	1.0–6.0	Netherlands	Overdevest *et al.* (2011), Kluytmans *et al.* (2013), Wu *et al.* (2013)
	4.0[1]–11.0[2]	Netherlands	Leverstein-Van Hall *et al.* (2011)
	8.8	Spain	Cortes *et al.* (2010)
	0.0	UK	Wu *et al.* (2013)
SHV-2	0.5	Germany	Kola *et al.* (2012)
	0.0[3]–5.0[4]	Netherlands	Stuart *et al.* (2012)
SHV-2A	2.1	Germany	Kola *et al.* (2012)
SHV-12	5.2	Belgium	De Jong *et al.* (2014)
	12.0	Denmark, import	Carmo *et al.* (2014)
	13.2–43.9	Germany	Kola *et al.* (2012), Laube *et al.* (2013), Belmar Campos *et al.* (2014), Blaak *et al.* (2015)
	16.28	Japan	Kawamura *et al.* (2014)
	0.0[2]–16.0[1]	Netherlands	Leverstein-Van Hall *et al.* (2011)
	3.0[3]–23.0[4]	Netherlands	Stuart *et al.* (2012)
	13.0–17.0	Netherlands	Overdevest *et al.* (2011), Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	7.8–82.7	Spain	Blanc *et al.* (2006), Egea *et al.* (2012)
	19.0	Switzerland	Geser *et al.* (2012)

[1]Chicken meat.
[2]Poultry.
[3]Organic farming.
[4]Conventional farming.

specific. Possible inhibitory effects on ESBL-producing bacteria are probably directed towards the bacteria, regardless the ability to produce ESBL or not. Nevertheless, antagonizing a bacterial family or genus like *E. coli*, which commonly harbor ESBL-genes, the prevalence of ESBL-producing bacteria might reduce subsequent to treatment with probiotics. This leads to the assumption, that antibacterial activity, competitive exclusion and the modulation of the immune system by probiotic strains may reduce ESBL-producing bacteria in broilers. Secretion of microbial substances such as organic acids, bacteriocins or hydrogen peroxide are examples for antibacterial activity. Organic acids, such as lactic acid, acetic acid and propionic acid, may contribute to a lower pH and thereby decrease the number of pathogenic bacteria (Williams, 2010; Alloui *et al.*,

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Table 5. Recent prevalence of CTX-M in Enterobacteriaceae in poultry (2003–2013)

Enzyme	Prevalence (%)	Country of sample collection	Reference
CTX-M	10.59–89.0	Germany	Laube *et al.* (2013), Reich *et al.* (2013)
	12.34	China	Zheng *et al.* (2012)
CTX-M-1 group	5.3	Spain	Cortes *et al.* (2010)
CTX-M-1	19.6–44.8	Belgium	Smet *et al.* (2008), De Jong *et al.* (2014)
	5.7	China	Zheng *et al.* (2012)
	8.6–37.5	Denmark	Agerso *et al.* (2014), Carmo *et al.* (2014)
	18.0–69.0	Germany	Kola *et al.* (2012), Wu *et al.* (2013), Belmar Campos *et al.* (2014), Blaak *et al.* (2015)
	11.6	Japan	Kawamura *et al.* (2014)
	28.4–69.0	Netherlands	Kluytmans *et al.* (2013), Wu *et al.* (2013), Huijbers *et al.* (2014)
	42.0[1]–56.0[2]	Netherlands	Stuart *et al.* (2012)
	49.0[3], [4]	Netherlands	Leverstein-Van Hall *et al.* (2011)
	1.6–3.2	Spain	Blanc *et al.* (2006), Egea *et al.* (2012), Overdevest *et al.* (2014)
	71.0	Switzerland	Geser *et al.* (2012)
	73.0–100.0	UK	Toszeghy *et al.* (2012), Wu *et al.* (2013)
CTX-M-2	1.7–7.8	Belgium	Smet *et al.* (2008), De Jong *et al.* (2014)
	1.7	Denmark, import	Carmo *et al.* (2014)
	0.34–1.1	Germany	Kola *et al.* (2012), Belmar Campos *et al.* (2014), Blaak *et al.* (2015)
	51.2	Japan	Kawamura *et al.* (2014)
	0.0[2]–7.0[1]	Netherlands	Stuart *et al.* (2012)
	1.0–9.0	Netherlands	Leverstein-Van Hall *et al.* (2011), Overdevest *et al.* (2012), Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
CTX-M-3	0.63	China	Zheng *et al.* (2012)
	4.7	Japan	Kawamura *et al.* (2014)
	6.6	UK	Toszeghy *et al.* (2012)
CTX-M-8	20.9	Japan	Kawamura *et al.* (2014)
CTX-M-9 group	64.9	Spain	Cortes *et al.* (2010)
CTX-M-9	7.28	China	Zheng *et al.* (2012)
	1.7	Belgium	De Jong *et al.* (2014)
	0.0	Germany	Wu *et al.* (2013)
	1.0–5.0	Netherlands	Kluytmans *et al.* (2013), Wu *et al.* (2013)
	0.0–14.1	Spain	Blanc *et al.* (2006), Egea *et al.* (2012)
	0.0	UK	Wu *et al.* (2013)
CTX-M-14	3.48	China	Zheng *et al.* (2012)
	5.9	Belgium	Smet *et al.* (2008)
	0.34–5.7	Germany	Belmar Campos *et al.* (2014)
	1.0–2.3	Netherlands	Overdevest *et al.* (2012), Kluytmans *et al.* (2013), Huijbers *et al.* (2014)
	45.3	Spain	Blanc *et al.* (2006)
CTX-M-15	0.32	China	Zheng *et al.* (2012)
	2.0	Belgium	Smet *et al.* (2008)
	0.0–0.34	Germany	Belmar Campos *et al.* (2014), Blaak *et al.* (2015)
	11.6	Japan	Kawamura *et al.* (2014)
	0.0–1.2	Netherlands	Overdevest *et al.* (2011), Kluytmans *et al.* (2013)
	0.0–3.8	Spain	Egea *et al.* (2012)
	17.0	UK	Toszeghy *et al.* (2012)
CTX-M-24	0.95	China	Zheng *et al.* (2012)
CTX-M-27	0.32	China	Zheng *et al.* (2012)
	0.34	Germany	Blaak *et al.* (2015)
CTX-M-32	1.1	Netherlands	Huijbers *et al.* (2014)
	1.9–8.1	Spain	Blanc *et al.* (2006), Egea *et al.* (2012)
CTX-M-55	4.75	China	Zheng *et al.* (2012)
CTX-M-65	0.5	Germany	Kola *et al.* (2012)
CTX-M-65	1.9	China	Zheng *et al.* (2012)
CTX-M-84	0.0	Netherlands	Kluytmans *et al.* (2013)
CTX-M-98	0.0	China	Zheng *et al.* (2012)
CTX-M-102	0.32	China	Zheng *et al.* (2012)
CTX-M-104	0.32	China	Zheng *et al.* (2012)
CTX-M-NT	3.3	UK	Toszeghy *et al.* (2012)

[1]Conventional farming.
[2]Organic farming.
[3]Poultry.
[4]Chicken meat.

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2013). By rivaling for nutrition and attaching to the intestinal mucosa, probiotic strains may counteract the advancement of pathogens in the gastrointestinal tract of poultry. Lactobacilli commonly apply this mechanism of competitive exclusion against pathogens like *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Williams, 2010; Alloui *et al.*, 2013). Increased production of antibodies and cytokines by immune cells as well as enhanced local immune response and morphologic changes in the intestines may contribute to the antagonistic effect by probiotics, enhancing the immune system (Smith, 2014). Treatment with lactobacilli might result in stabilized tight junctions and stimulate the expression of mucins, reducing the adherence of pathogens to the epithelial cells in the intestines. A lower permeability and an enhanced local barrier, subsequent to these morphological alterations, may antagonize the uptake of pathogens (Otte and Podolsky, 2004; Doron and Gorbach, 2006). Furthermore, goblet cells, liable for local defense and reparation of the epithelium, might increase in the presence of probiotics as well (Smith, 2014). Prebiotics and synbiotics, a combination of pro- and prebiotics, may enhance the antagonistic effects of probiotics even further (Awad *et al.*, 2009; Huyghebaert *et al.*, 2011; Alloui *et al.*, 2013). As these mechanisms to combat pathogens may implement potential lethal threats on ESBL-producing bacteria the consequence to such stress must be considered (Boor, 2006). Genes encoding for ESBL are frequently located on plasmids, which may be transferred to other *Enterobacteriacea* by conjugation (Händel *et al.*, 2015; Yamaichi *et al.*, 2015; Porse *et al.*, 2016). Whether the stress induced by potential reduction measurements may induce higher conjugation frequencies has yet to be investigated.

Due to the correlation between antibiotic, zinc and copper resistance, a reduction of zinc and copper contents in animal feed may help to combat the prevalence of ESBL-producing bacteria in poultry. Already, the European Food Safety Authority (EFSA) recommended lower maximum copper contents in piglet and cattle feed in August 2016 (https://www.efsa.europa.eu/en/press/news/160809a). Correspondingly, the Committee for Medicinal Products for Veterinary Use (CVMP) recommended to withdraw veterinary medicinal products containing zinc oxide from the market in December 2016 (http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2016/12/news_detail_002661.jsp&mid=WC0b01ac058004d5c1).

Nutrition has a major impact on the gastrointestinal composition and antibiotic resistance in people and animals. The diversity and quantity of genes encoding for antibiotic resistance obtained from intestinal bacteria from obese children decreased due to an alteration of the dominant microbial fermentation source from protein to carbohydrates. Especially mechanisms for target alteration and efflux pumps were affected (Wu *et al.*, 2016).

Besides from optimizing feed and feed additives, an ESBL-free environment is necessary to keep poultry flocks free from new infections. However, this is almost impossible due to the high prevalence and spread of ESBL-producing bacteria (Hiroi *et al.*, 2012a). Nevertheless, high biosecurity, a low number of persons entering the stables as well as thorough cleaning and disinfection, may reduce the prevalence of ESBL-producing bacteria in poultry farms. Limiting the number of chicks' suppliers may also reduce prevalence and diversity of ESBL types in poultry farms (Mo *et al.*, 2016). In order to avoid international carryover, traded animals must not carry ESBL-producers. Therefore, an ESBL-reduction strategy should include all levels of the poultry production pyramid to be successful (Stuart *et al.*, 2012). Producers of grandparent chicks should aim for ESBL-free flocks and regular controls.

Conclusions

CTX-M-1, TEM-52 and SHV-12 are the extended-spectrum ß-lactamases most frequently detected in poultry. The high prevalence of ESBL-producing bacteria in poultry provides a global challenge, which should be addressed with preventive reduction measurements on all levels of the poultry production system, the environment and dietary factors.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1466252317000020.

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