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Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective

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Abstract

The possible zoonotic spread of antimicrobial-resistant bacteria is controversial. This review discusses global molecular epidemiological data combining both analyses of the chromosomal background, using multilocus sequence typing (MLST), and analyses of plasmid (episomal) extended-spectrum β-lactamase (ESBL)/AmpC genes in *Escherichia coli* present in humans and animals. For consideration of major epidemiological differences, animals were separated into livestock and companion animals. MLST revealed the existence of ESBL-producing isolates thoughout the *E. coli* population, with no obvious association with any ancestral EcoR group. A similar distribution of major ESBL/AmpC types was apparent only in human isolates, regardless of their geographical origin from Europe, Asia, or the Americas, whereas in animals this varied extensively between animal groups and across different geographical areas. In contrast to the diversity of episomal ESBL/AmpC types, isolates from human and animals mainly shared identical sequence types (STs), suggesting transmission or parallel micro-evolution. In conclusion, the opinion that animal ESBL-producing *E. coli* is a major source of human infections is oversimplified, and neglects a highly complex scenario.

Keywords: AmpC, companion animals, *Escherichia coli*, extended-spectrum β-lactamase, livestock, multilocus sequence typing
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Introduction

Escherichia coli is a particularly complex species, having diversified into commensals of the intestinal microbiota and pathogenic strains, grouped into pathotypes of partly zoonotic intestinal pathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC) [1,2]. Only recently has the role of ExPEC in severe infections received attention, both from a veterinary clinical perspective and regarding their zoonotic potential [2–5].

The increase in antimicrobial-resistant (AMR) bacteria of animal origin resembles the process in humans a decade ago [6–8]. Since the late 1990s, extended-spectrum β-lactamase (ESBL)-producing and AmpC β-lactamase-producing *Enterobacteriaceae*, in particular *E. coli*, have emerged globally. Whereas early ESBLs from humans mainly evolved from TEM and SHV β-lactamases, the significance of CTX-M-type enzymes has increased over the last decade. Currently they represent the most common and still rising ESBL type in humans [9–11]. Since 2000, the European Antimicrobial Resistance Surveillance Network has reported a steady increase in the rates of invasive *E. coli* and *Klebsiella pneumoniae* isolates resistant to third-generation and fourth-generation cephalosporins. ESBLs confer resistance to oxyiminocephalosporins, and often express a multidrug-resistant phenotype, leaving only limited therapeutic options [10]. In addition, plasmid-mediated AmpC β-lactamases (e.g. CMY and

CIT) and carbapenemases—so far only seen in human isolates (e.g. KPC, NDM, and OXA-48)—are contributing to the worrying situation regarding antimicrobial resistance, as they mediate resistance against almost all available β-lactam agents [2].

Initially, ESBL/AmpC-producing bacteria were only observed in human medical practice, but the recent observation of these bacteria, first in companion animals and increasingly in livestock, has initiated monitoring studies concentrating on livestock [6,7]. ESBL/AmpC-producing *E. coli* isolates are now being found in increasing numbers in food-producing animals [7,12], leading to the hypothesis that animals might become infection sources or even reservoirs—the natural persistent source of infection—contributing to the spread of these bacteria. Companion animals are putatively involved in this vicious cycle, as they often live in close contact with their owners. As the term reservoir is used imprecisely, not distinguishing between a temporary and a persistent infection source, it is hard to follow the literature [6,8]. Fig. 1 illustrates the transmission pathways of AMR bacteria and the possible impact of different habitats.

This review is mainly dedicated to the habitat 'animal' (companion animals and livestock) and its complex interplay with the habitat 'human'. Published data on ESBL/AmpC-producing *E. coli* and other *Enterobacteriaceae* are summarized and evaluated, with a focus on available molecular epidemiological and phylogenetic data for both the chromosomal background and acquired episomal β-lactamase types. The conclusion is that we are far from giving simple and exhaustive answers to the questions concerning the precise role of animals in the transfer of ESBL/AmpC-producing *E. coli* to humans. However, to the best of our knowledge, this is currently the most comprehensive consideration of the occurrence of ESBL/AmpC types and their linkage to phylogenetic *E. coli* lineages, with respect to host and geographical distribution.

General Aspects of the Use of Antimicrobials and Transfer of AMR Bacteria

The prudent use of antimicrobials in veterinary and human medicine is a prerequisite for the successful treatment of serious infectious diseases, and is thus also a matter of animal welfare. β-Lactam antibiotics, especially cephalosporins, also select for ESBL-producing *Enterobacteriaceae* in animals, enhancing the risk of the zoonotic transmission of ESBL-carrying bacteria and plasmids [13–15]. However, increases in the numbers of ESBL/AmpC-producing strains of *E. coli* and *Salmonella* spp. also occur without any prior use of cephalosporins [15,16]. The reasons for this are not fully understood, as co-selection and co-resistance do only contribute partially. In companion animals, as in humans, treatment with fluoroquinolones has led to an increase in the number of

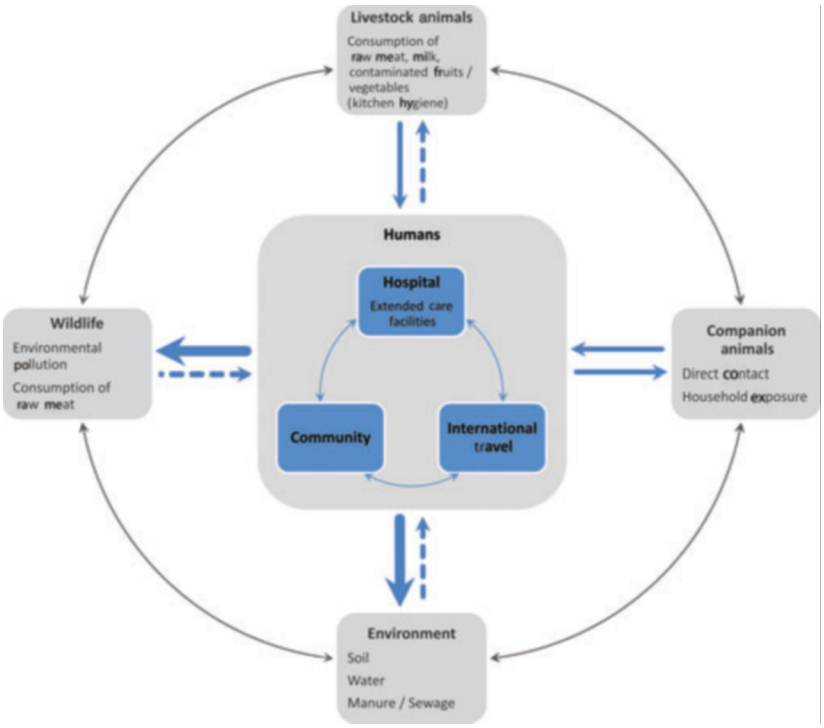

FIG. 1. Diagram illustrating the transmission pathways of antimicrobial resistance among different habitats.

multiresistant strains [17], and its use on farms would be expected to exert selective pressure [13]. In contrast, the impacts of different steps in livestock production needs consideration. In chickens, the percentages of ESBL-producing *E. coli* decrease from grandparents to parents and again to the production level, showing the complexity of the influence of antimicrobial treatment and the maintenance of AMR bacteria in food-producing animals [18].

The use of antimicrobials in livestock needs to be distinguished from that in companion and exotic animals, for two reasons: (i) in general, companion animals live as individuals or in small groups, leading to individual therapeutic interventions, whereas livestock, in general, is kept in larger groups, so population-based therapeutics are mostly appropriate; (ii) companion animals often live in intimate contact with their owners, enabling the spread of multiresistant and zoonotic agents between owners and animals much more efficiently, whereas livestock is raised for the production of food, raising safety issues, in particular in terms of possible food contamination [19].

In addition, the infection epidemiology of humans, companion animals and livestock differs significantly, suggesting different risk factors [6,19–21]. The lack of comparative monitoring or surveillance studies incorporating both human and veterinary medicine in the same geographical area hinders the identification of risk factors. Furthermore, the impact of antimicrobials and resistant bacteria in companion animals has so far been largely neglected [22]. Despite this lack of knowledge, policies aimed at reducing the amount of multiresistant bacteria have been implemented. In the veterinary area, these are (i) monitoring programmes, which are mostly restricted to livestock, (ii) an EU-wide ban on the use of growth promoters, initiated in 1999; and (iii) guidelines for the prudent use of antibiotics. We are not aware of true surveillance studies, in that the results of monitoring directly impact on the later use of antimicrobials. Another issue is the use of distinct antimicrobial classes, both in veterinary and in human medicine, and the prioritization of each class in the respective area. A catalogue of criteria identified the most critical important antimicrobials as cephalosporins (third and fourth generation), macrolides, and quinolones. Basically, these should be used in human medical practice only [23]. Again, these recommendations neglect the differences between companion/exotic animals and livestock.

The availability and usage of antimicrobials differ between livestock and companion animals. For small animal populations or for rare infectious diseases, effective antimicrobials may simply not be available, owing to lack of approval. Thus, there is a need for off-label use under specific circumstances, meaning that, if no medicine is authorized, veterinarians have to use a particular antimicrobial that is approved in another country or in the medical area [19]. Any other practice is in conflict with animal welfare.

Distribution of ESBL/AmpC-producing *E. coli* in Livestock and Companion Animals

Until the 1990s, the majority of ESBLs identified in human clinical isolates were SHV or TEM types [2]. Almost one decade later, ESBL and AmpC β-lactamases have emerged worldwide, and CTX-M enzymes have now become the most widespread type of ESBL [9,10]. A number of studies have described the occurrence of *E. coli* producing ESBL/AmpC in food-producing animals, and strains relevant to human health are increasingly being isolated also from companion animals [6,7,12,24,25]. The most frequent genes associated with this resistance among both groups of animals encode various CTX-M enzymes, followed by $bla_{\text{TEM-52}}$ and $bla_{\text{SHV-12}}$; other TEM and SHV types are also observed (Table S1) [6,7,25]. Among the AmpC β-lactamases, $bla_{\text{CMY-2}}$ is by far the most common, other types having only rarely been identified (Table S1) [12,25].

Although the first ESBL in humans was identified during a *K. pneumoniae* outbreak in a German hospital in 1982, one of the first clinical ESBLs observed in animals dates back to 2000, from an SHV-12-producing *E. coli* isolate from a dog with recurrent urinary tract infection [26]. The earliest description of poultry as carriers of ESBLs was by Briñas *et al.* [27], who observed CTX-M-14-producing, SHV-12-producing and CMY-2-producing *E. coli* in the faeces of healthy chickens in Spain between 2000 and 2001. At almost the same time, in 1999–2002, the isolation of bacteria carrying CTX-M-14, CTX-M-2 and CMY-2 from healthy poultry was reported in Japan [28]. Studies documenting the occurrence of various ESBL types in pigs and cattle followed soon after [29].

An overview of studies performed on the occurrence and the nature of β-lactamase types in various animal species and humans, respectively, is provided in Tables S1 and S2. Although by no means complete, these data indicate the development of AMR bacteria roughly within the last decade. Based on this, the distribution of ESBL/AmpC enzymes among *E. coli*, as the most studied bacterial organism, is illustrated with respect to geographical and host origin in Fig. 2. Although most studies are still dedicated to human samples (Table S2), the second most numerous reports (Table S1; 52 cited publications) concern poultry. Most of these studies are from European countries, with a prevalence of various ESBL/AmpC types ranging between 0.6% and 44.7% (Table S1; Fig. 2). There are also quite a number of reports from Asia,

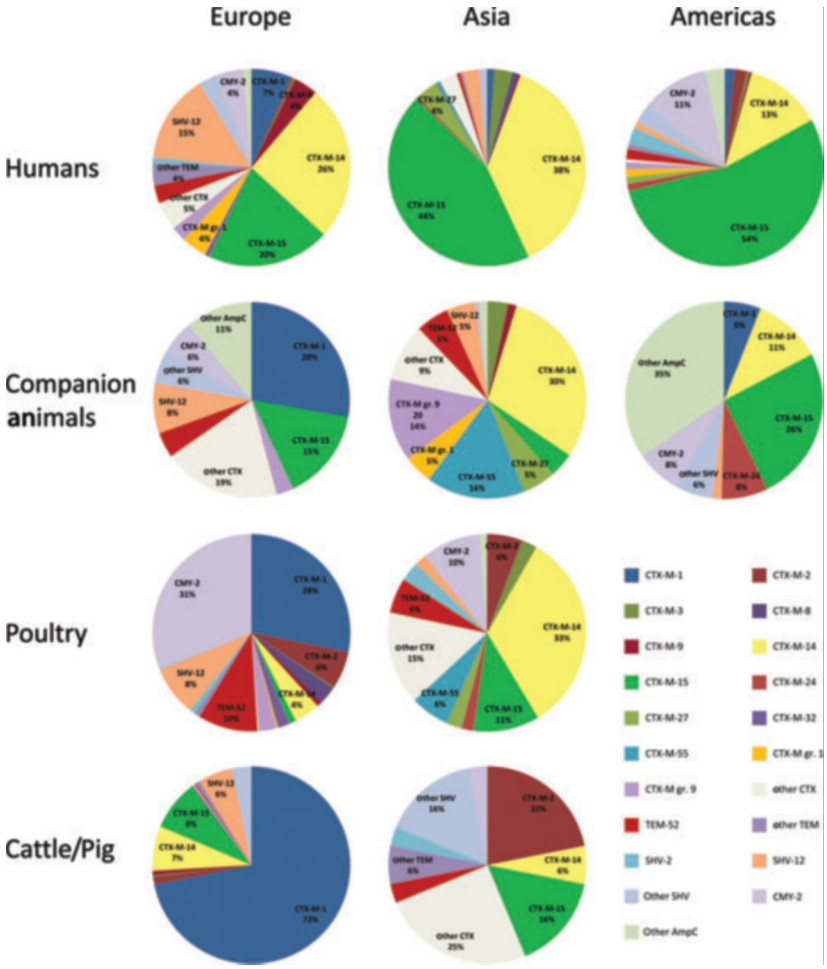

FIG. 2. Spatial and host distribution of *Escherichia coli* extended-spectrum β-lactamase (ESBL)/AmpC types with regard to data and the references given in Tables S1 and S2. With the exception of the category 'Companion animals—Asia', all pie charts presented are based on a minimum number of eight studies.

with rates from 1.7% to 11.8% of ESBL/AmpC-producing *E. coli* and *Salmonella* spp. in poultry. Data from North and South America and from African countries seem to be rather limited, and thus are not included in Fig. 2. However, these studies ascertain the global occurrence of ESBL/AmpC-producing bacteria in poultry (Table S1). Other food-producing animals, including cattle and pigs, are also either colonized or infected by such bacteria to varying degrees (Table S1).

The occurrence of ESBL-producing bacteria in companion animals has been neglected for a long time, but a growing burden is now being observed, especially in dogs, cats, and horses, with most of the 30 studies listed in Table S1 having been published within the past 5 years [6,8,30–32]. Initial data on the prevalence, in particular, of ESBL-producing *E. coli* from companion animals indicate high carriage and infection rates (Table S1). Both diseased and healthy animals are affected, raising animal welfare and public health issues. This deserves more attention in future surveillance, as the extent and genetic basis of AMR bacteria in companion animals are poorly understood.

ESBL/AmpC Types Reported in *E. coli* From Livestock and Companion Animals

It is far too early to judge the direction of transmission of ESBL/AmpC-producing bacteria between humans and animals in general. An important part of the global success of, for example, CTX-M ESBLs is the wide dissemination of particular plasmids or bacterial clones [9]. Thus, apart from a common ancestry of ESBL carriers, a central issue is the sharing of identical plasmid β-lactamase types by humans and animal hosts, which has recently been reviewed [15,33]. Therefore, this review will focus on ESBL/AmpC types only.

Fig. 2 shows the distribution of ESBL/AmpC types in *E. coli* originating from four different habitats and three different geographical regions. Irrespective of the group considered, two to a maximum of three ESBL/AmpC types always predominate, without overall congruence. The most frequent types are CTX-M-1, CTX-M-14, CTX-M-15, SHV-12, and CMY-2. A similar distribution of ESBL/AmpC types is only

seen in humans, CTX-M-14 and CTX-M-15 being the major types regardless of geographical origin. One type is broadly disseminated among animals in Europe, namely CTX-M-1 (companion animals, 28%; poultry, 28%; cattle and pigs, 72%), whereas it is only rarely reported in other regions and habitats. In general, CTX-M-14 is among the most prevalent β-lactamase types in companion animals and poultry in Asia (30–33%), and to a lesser extent in cattle and pigs (14%). It is less prevalent in livestock (4–7%) in Europe, and is even absent in companion animals.

CTX-M-15, which has spread pandemically in humans [9], was only detected incidentally in poultry in European countries, whereas companion animals (15%) and cattle/pigs (8%) are frequently associated with this type. In Asian and American countries, this enzyme is present in bacteria from all groups of animals studied. Although ESBL types are highly diverse, one episomal AmpC variant, CMY-2, has been described in all areas and hosts investigated (Table S1; Fig. 2), its frequency ranging from 2% to 31%.

Hence, a similar distribution of ESBL types is only found in humans; for the animal groups, the observed patterns are highly diverse and incongruent. The same is true in terms of geography. With the exception of CTX-M-14 in Asia, none of the ESBL types predominates over all other animal and human groups in one particular area.

CTX-M-1, as a major ESBL type in cattle and pigs in Europe, amounts to 72% of all ESBLs, and is also frequent in poultry and companion animals. CTX-M-1-producing *E. coli* accounts for 7% of all types identified among humans in Europe only. Nevertheless, two recent studies from The Netherlands identified CTX-M-1 as the most prevalent ESBL type shared by human patients, healthy carriers, poultry, and retail chicken meat, suggesting recent cross-transmission between human and avian hosts [24,34]. A relationship between contamination of chicken meat and the appearance of ESBL genes in humans, and thus transmission of ESBL-producing *E. coli* from poultry to humans, was also suggested, although without unequivocal proof. The literature gives limited evidence for the spread of ESBL/AmpC-carrying organisms via direct contact with livestock [15]. In summary, the collective data of the available studies reveal considerable differences in ESBL types between poultry and humans in Europe, leaving the question open as to what extent livestock contributes to the spread of ESBL in humans.

Human and Animal ESBL/AmpC-producing *E. coli* Share Identical Phylogenetic Lineages

Partial overlap of β-lactamase types and/or plasmid scaffolds from human ESBL/AmpC-producing *E. coli* with those of animal origin might indicate ongoing interspecies transmission, but also parallel independent micro-evolution. A sound analysis of the putative zoonotic nature of such isolates is only possible by additional typing of the chromosomal background. Multilocus sequence typing (MLST) reflects micro-evolution of the *E. coli* core genome, providing a true sketch of the population structure of this bacterial species [35].

In 2008, MLST investigation revealed a pandemic clone, B2-O25b:H4-ST131-CTX-M-15, with high extraintestinal virulence, causing urinary tract infections, bacteraemia, urinary sepsis, and neonatal sepsis [36,37]. Sequence type (ST)131 is the most studied phylogenetic lineage in terms of antimicrobial resistance in *E. coli* (Figs 3 and 4). Soon after the first discovery of ESBL-producing ST131 *E. coli* in human clinical isolates, it disseminated to various animal species, including poultry, cattle, pigs, wildlife, and companion animals, and several studies suggested transmission from poultry or retail chicken, but without clear evidence [11,24,38–40].

With the increasing awareness that companion animals suffer from extraintestinal infections caused by *E. coli* phylotypes identical to those that infect humans, the recovery of ST131, in fact a typical ExPEC lineage, from this animal group is reasonable [11,41]. Accordingly, we recently confirmed the presence of CTX-M-15-producing and SHV-12-producing ST131 *E. coli* in dogs, cats, and one horse [39]. Ongoing typing of clinical strains from companion animals (unpublished data) have demonstrated similar rates of ST131 in the past 2 years, whereas the diversity of CTX-M types is increasing over time and with the number of isolates (Table 1). This corroborates data indicating that, in the past few years, although still mainly associated with CTX-M-15, human clinical ST131 strains have acquired various ESBL genes linked to plasmids of growing complexity, as assessed by Inc/rep typing and plasmid MLST, and have diversified in terms of other transferable resistance elements [37].

ST131 ancestrally derived from EcoR B2, which contains highly virulent ExPEC, and its combination of multiresistance and virulence is discussed as likely reason for the pandemic success of this ST [2,37,41]. The paradigm that maintenance of antibiotic resistance and high levels of virulence ultimately lead to loss of bacterial fitness is false for ST131, corroborating numerous reports on bacteria that have ameliorated the costs of resistance, e.g. by compensatory mutations [41,42]. However, attributing the success of ST131 simply to its virulence would draw a distorted picture [43]. If virulence is a major driver of the emergence and supposed repeated selection of AMR ST131 and local variants, why, then, have other highly virulent ExPEC lineages failed to benefit from this combination? For example, B2-ST complex (STC)95,

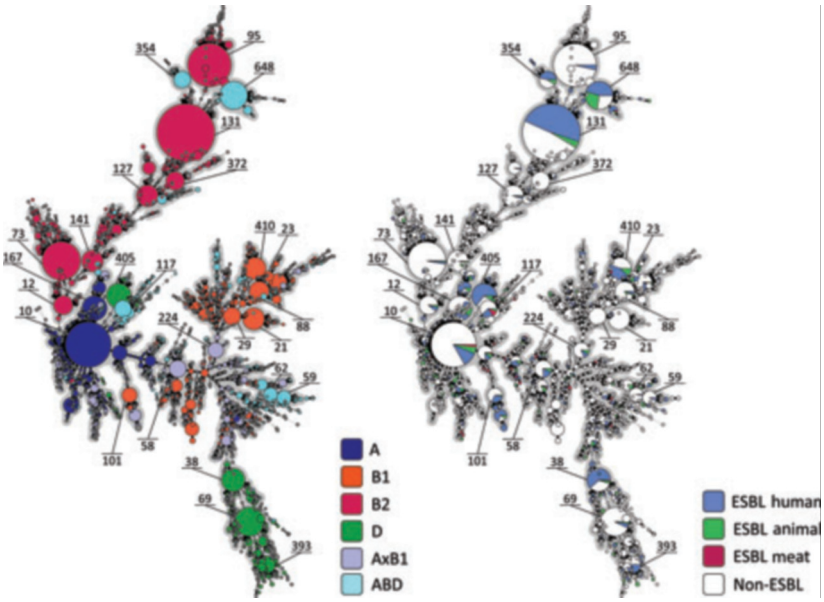

FIG. 3. Minimum spanning tree (MSTree, calculated with Bionumerics 6.6 (Applied Maths, Sint-Martens-Latem, Belgium)) showing the population structure of 7766 *Escherichia coli* strains based on allele sequence combinations of genes *adk*, *fumC*, *purA*, *recA*, *gyrB*, *icd*, and *mdh*. The distribution of the phylogenetic/ancestral groups, as determined by structure analysis and based on the concatenated sequences of the seven allele sequences, is shown on the left, and the distribution of extended-spectrum β-lactamase (ESBL)/AmpC/NDM-1-producing *E. coli* (n = 1863) in the background of the *E. coli* population is presented on the right. For multilocus sequence typing (MLST) data and information about the ESBL status of the strains, see the MLST database (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli/) (Table S3 (including relevant references), Data S1 (providing available references for MLST data), and own unpublished results.

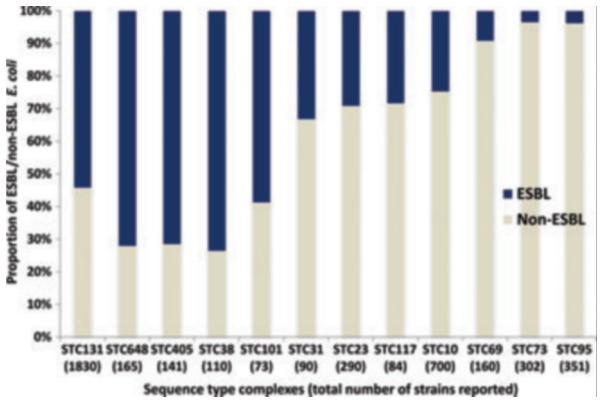

FIG. 4. Proportion (%) of extended-spectrum β-lactamase (ESBL)/AmpC/NDM-producing *Escherichia coli* among the total number of strains recorded among various sequence type complexes (STCs).

which is currently represented by 351 strains either deposited in the web-hosted database (http://mlst.ucc. ie/mlst/mlst/dbs/Ecoli/) or reported in publications, represents one of the most virulent groups of globally distributed ExPEC strains [4,5,35,44]. However, only marginal proportions (4%) of STC95 strains harbour ESBL genes (Table 1; Fig. 4). This is also the case for B2-STC73 (3.6%) and for other ExPEC-B2 strains known to express a comparable set of ExPEC-related virulence determinants as observed in ST131 strains, such as STC127 (4.3%), STC141 (1.1%), and STC372 (0%) (proportions calculated according to the MLST database, data given in Table 1, and our own unpublished data) [43]. Humans and poultry are the main hosts of STC95 strains, accounting for 74.4% and 24.2% of all recorded strains, respectively. Septicaemia is the main clinical outcome that is relevant for human patients and poultry flocks, and a zoonotic nature of avian pathogenic *E. coli* of STC95 is frequently discussed [45]. If antimicrobial agents favoured the selection of ESBL/AmpC-producing bacteria in the poultry host, this would, in turn, lead to an increased risk of humans acquiring these strains via consumption of contaminated food. Why is STC95, which is such a well-established and clinically successful phylogenetic lineage, less prone to such an event than its B2-ExPEC 'relative' STC131? Clearly, future detailed investigations are needed to answer this question.

Taking a closer look at the distribution of ancestral groups and the occurrence of ESBL/AmpC-producing bacteria in the phylogenetic background of >7700 *E. coli* strains shown in Fig. 3, it becomes apparent that multiresistant strains are dispersed over the entire population. Different STs have recently been identified among ESBL-producing *E. coli* linked to various ancestral groups, such as D (STC38, ST405, and

TABLE 1. Phylogenetic lineages associated with the spread of extended-spectrum β-lactamase (ESBL), and AmpC β-lactamase genes in *Escherichia coli* according to the multilocus sequence typing database (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli), published data (for references see Table S3), and own unpublished results

Sequence type complex (STC) and sequence type (ST) (no. of strains reported)	Type of β-lactamase gene	Confirmed presence (x) in:				Spread in:	
		Companion animals	Livestock animals	Wildlife	Humans	Animals	Humans
STC131 (*n* = 995)	$bla_{CTX\text{-}M\text{-}3,9,14,15,27,32}$	x[a]	x	x	x	Global[a]	Global
ST131 (B2) (*n* = 983)	$bla_{SHV\text{-}5,7,12}$						
Nine other STs (*n* = 12)	$bla_{CIT\ type}$						
	$bla_{NDM\text{-}1}$						
STC648 (*n* = 119)	$bla_{CTX\text{-}M\text{-}14,15,32}$	x[a]	x[a]	x	x	Global[a]	Global
ST648 (ABD) (*n* = 101)	$bla_{CIT\ type}$						
Ten other STs (*n* = 18)	$bla_{NDM\text{-}1}$						
STC405 (*n* = 101)	$bla_{CTX\text{-}M\text{-}3,14,15}$	x[a]		x	x	Europe[a]	Global
ST405 (D) (*n* = 94)	$bla_{CTX\text{-}M\ group\ 1}$						
Six other STs (*n* = 7)	$bla_{CIT\ type}$						
	$bla_{NDM\text{-}1}$						
STC38 (*n* = 81)	$bla_{CTX\text{-}M\text{-}1,9,14,15,27}$	x[a]	x		x	Europe[a]	Global
ST38 (D) (*n* = 68)	$bla_{OXA\text{-}48}$						
ST315 (D) (*n* = 13)	$bla_{NDM\text{-}1}$						
STC101 (*n* = 43)	$bla_{CTX\text{-}M\text{-}14,15}$	x[a]		x	x	Europe[a]	Europe, Asia, Africa
ST101 (B1) (*n* = 21)	$bla_{CTX\text{-}M\ group\ 1}$						
ST359 (AxB1) (*n* = 17)	$bla_{CIT\ type}$						
Two other STs (*n* = 5)	$bla_{NDM\text{-}1}$						
STC31 (*n* = 30)	$bla_{CTX\text{-}M\text{-}14,15}$		x[a]		x	Germany[a]	Global
ST393 (D) (*n* = 28)							
Two other STs (*n* = 2)							
STC23 (*n* = 85)	$bla_{CTX\text{-}M\text{-}1,3,14,15}$	x	x	x	x	Europe[a]	Global
ST410 (B1) (*n* = 42)	$bla_{SHV\text{-}12,44}$						
ST23 (B1) (*n* = 10)	$bla_{NDM\text{-}1}$						
ST88 (B1) (*n* = 10)	$bla_{TEM\text{-}52}$						
ST90 (B1) (*n* = 9)							
11 other STs (*n* = 14)							
STC117 (*n* = 18)	$bla_{CTX\text{-}M\text{-}1,2,14,15}$	x[a]	x		x	Europe[a]	Europe, Asia
ST117 (ABD) (*n* = 16)							
Two other STs (*n* = 2)							
STC10 (*n* = 174)	$bla_{CTX\text{-}M\text{-}1,2,14,15}$	x[a]	x[a]	x	x	Global[a]	Global
ST10 (A) (*n* = 56)	$bla_{SHV\text{-}5,12}$						
ST167 (A) (*n* = 33)	$bla_{TEM\text{-}52}$						
ST617 (A) (*n* = 25)							
47 other STs (*n* = 60)							
STC69 (*n* = 15)	$bla_{CTX\text{-}M\text{-}1\text{-}14}$	x[a]	x[a]	x	x	Europe[a]	Global
ST69 (D) (*n* = 12)							
ST106 (D) (*n* = 3)							
STCs of non-STC131 B2 lineages only sporadically associated with the spread of ESBL-producing *E. coli*							
STC95 (*n* = 14)	$bla_{CTX\text{-}M\text{-}3,14,15}$				x	–	Global
ST95 (B2) (*n* = 10)							
Three other STs (*n* = 4)							
STC73 (*n* = 11)	$bla_{CTX\text{-}M\text{-}14,15}$	x[a]	x[a]		x	Germany[a]	Global
ST73 (B2) (*n* = 6)							
ST638 (B2) (*n* = 4)							
ST458 (B2) (*n* = 1)							

[a]According to our own unpublished data, marked STCs also occur in various animal species throughout Europe: STC648 ($bla_{CTX\text{-}M\text{-}1,3,14,15,61;\ group\ 9}$) and STC23 ($bla_{CTX\text{-}M\text{-}1,2,14,15,32}$) in dogs, cats, horses, and cattle; STC10 ($bla_{CTX\text{-}M\text{-}1,2,14,15,32}$) in dogs, cats, horses, cattle, pigs, and birds; STC405 ($bla_{CTX\text{-}M\text{-}3,14,15,61;\ group\ 9}$) and STC131 ($bla_{CTX\text{-}M\text{-}1,2,14,15,27,55}$) in dogs, cats, horses, and cattle; STC38 ($bla_{CTX\text{-}M\text{-}3,14}$) in dogs; STC69 ($bla_{CTX\text{-}M\text{-}1,15}$) in dogs and cattle; STC73 ($bla_{CTX\text{-}M\text{-}1}$) in cats and cattle; STC117 ($bla_{CTX\text{-}M\text{-}1}$) in cats; STC101 ($bla_{CTX\text{-}M\text{-}14}$) in cats and dogs.

STC69), A (ST10, ST167, and ST617), B1 (ST410), and hybrid ABD (ST648) (Tables 1 and S3). However, similar to the situation observed among group B2 complexes, the accumulation of multiresistant strains in certain non-B2 STCs known to cover strains of intermediate (ancestral group D, ABD) or low extraintestinal virulence (groups B1 and A) differs noticeably (Fig. 4) [35,41]. Although the data presented are just a snapshot of a highly complex and dynamic process, they highlight the fact that some groups, such as STC648 (ancestral group ABD; 71.1% ESBL/AmpC-producing strains), STC405 (D; 71.6%), and STC38 (D; 73.6%), have a higher proportion of multiresistant strains than others, e.g. STC69 (D; 9.4%), STC23 (B1; 29.3%), and STC10 (A; 24.9%). With respect to the varying virulence gene content of the phylogenetic lineages discussed, one might speculate about common, so far unidentified, genetic factors located in the core genome that give rise to the increased ability of such strains to acquire and retain resistance plasmids. As the clinical relevance of most of the non-B2 ExPEC groups has significantly increased only with the recognition of their multiresistant phenotype (Table 1), we are just at the very beginning of unravelling the underlying mechanisms.

As strains of various clonal groups are circulating widely, they are prone to acquire whatever resistance plasmids are

locally prevalent [46]. Accordingly, although distributions may differ from case to case, an exclusive linkage of one *bla* gene or a distinct host with a certain ST or STC is not evident (Table 1). Given that animal strains constitute only a low proportion (<20%) of ESBL-producing *E. coli* isolates analysed with MLST, it is even more intriguing that these isolates mainly share identical phylotypes with human strains, suggesting frequent and continuous interspecies transmission. However, only more discriminative typing tools can provide further support for this hypothesis. The schedule of events, i.e. the first appearance of ESBL-producing bacteria in the medical context and the earlier recovery of prominent phylotypes, such as ST131, from humans, might (mis)lead us into thinking that the initial transfer of multiresistant strains was from humans to animals. The molecular epidemiological data are just as tentative as the growing discussion about livestock as the primary infection source of such bacteria.

Conclusions

We have discussed global molecular epidemiological data on ESBL/AmpC-producing *E. coli* by comparing phylotypes with episomal resistance genes in different hosts. Owing to the lack of data on incompatibility groups of plasmids in concert with MLST and ESBL/AmpC types, these were not included. Also, data from single continents were excluded because of a limited number of publications. Most importantly, the similarity of major ESBL/AmpC types and STs in humans regardless of their geographical origin points towards person-to-person transmission as the most important route of antimicrobial resistance distribution. Whether the initial spread of such strains from humans as original carriers was the source of the latter enrichment of CTX-M-producing *E. coli* in animals remains unknown. However, the primary occurrence of CTX-M-1 points in this direction, at least in Europe.

Apart from this, the analysed data raise more questions than answers. Defining routes and directions of transmission is possible, as all of the molecular methods needed are at hand, up to whole genome next-generation sequencing. However, these methods need to be applied in parallel in medical and veterinary areas. If harmonized protocols are used within a given time and geographical area, the flow of ESBL-producing *E. coli* can be traced. At the same time, detailed knowledge of antimicrobial usage in humans, livestock and companion animal populations should be recorded. As such data are currently missing, improved parallel monitoring and surveillance programmes are desperately needed.

Furthermore, a more sound understanding of horizontal gene flow between different *E. coli* and related *Enterobacteriaceae* strains is needed. Our collection of MLST data of >7700 *E. coli* strains revealed plasmids encoding ESBL/AmpC genes over the whole *E. coli* population, showing highly promiscuous gene transfer, which can hardly be controlled. Would competitive exclusion with probiotics be an option to reduce intestinal ESBL/AmpC-producing strains? Or could we even vaccinate against these bacteria? To tackle this challenge, we also need a more comprehensive understanding of the mechanisms of colonization as well as antimicrobial resistance. Currently, the increase in ESBL/AmpC-producing *E. coli* is mainly considered to be a consequence of the overuse of antimicrobials. Although we do not argue against this obvious fact, which makes the prudent use of antimicrobials mandatory, the additional factors involved need to be unravelled. Pandora's Box has been opened, but rather than continuing with the 'name, shame and blame' approach, it is more straightforward to encourage interdisciplinary and novel strategies in the spirit of 'One Health'.

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Transparency Declaration

The authors have no conflicts of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary document to Fig. 3.

Table S1. Presence of ESBL/AmpC-producing *Enterobacteriaceae* spp. in animals organized according to animal groups (companion animals (cats and dogs; horses) and livestock animals (cattle and sheep; pigs; poultry)], geographical area (Europe; Asia/Oceania; North America; South America; Africa), and date of publication.

Table S2. Presence of ESBL/AmpC-producing *Enterobacteriaceae* spp. in humans organized according to geographical

area (Europe; Asia/Oceania; North America; South America; Africa) and date of publication.

Table S3. Phylogenetic lineages associated with the spread of ESBL/AmpC genes in *Escherichia coli* according to the MLST database (http://mlst.ucc.ie/mlst/mlst/dbs/Ecoli/), cited references, and our own unpublished results.

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