Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution?

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Wildlife is normally not exposed to clinically used antimicrobial agents but can acquire antimicrobial resistant bacteria through contact with humans, domesticated animals and the environment, where water polluted with feces seems to be the most important vector. *Escherichia coli*, an ubiquitous commensal bacterial species colonizing the intestinal tract of mammals and birds, is also found in the environment. Extended-spectrum beta-lactamases producing *E. coli* (ESBL-*E. coli*) represent a major problem in human and veterinary medicine, particular in nosocomial infections. Additionally an onset of community-acquired ESBL-*E. coli* infections and an emergence in livestock farming has been observed in recent years, suggesting a successful transmission as well as persistence of ESBL-*E. coli* strains outside clinical settings. Another parallel worldwide phenomenon is the spread of ESBL-*E. coli* into the environment beyond human and domesticated animal populations, and this seems to be directly influenced by antibiotic practice. This might be a collateral consequence of the community-onset of ESBL-*E. coli* infections but can result (a) in a subsequent colonization of wild animal populations which can turn into an infectious source or even a reservoir of ESBL-*E. coli*, (b) in a contribution of wildlife to the spread and transmission of ESBL-*E. coli* into fragile environmental niches, (c) in new putative infection cycles between wildlife, domesticated animals and humans, and (d) in problems in the medical treatment of wildlife. This review aims to summarize the current knowledge on ESBL-*E. coli* in wildlife, in turn underlining the need for more large scale investigations, in particular sentinel studies to monitor the impact of multiresistant bacteria on wildlife.

Keywords: ESBL, wildlife, wild birds, rodents, multiresistance

INTRODUCTION

The mere occurrence of antimicrobial resistance and corresponding resistance genes in the environment is an ancient phenomenon which results from the simple fact that most of the antimicrobial substances currently in use are based on natural precessors produced by soil bacteria like *Streptomycetes* (D'Costa et al., 2011). The function of these precessors of modern day antibiotics was presumably more related to microbial competition for an ecological niche, and thus is very distinct from the "weapon-shield" role they play in clinical settings today (Martinez, 2009a,b; Allen et al., 2010). Nevertheless the increase in non-intrinsic antimicrobial resistance in pathogenic bacteria started after the introduction of antibiotics in medicine some 60 years ago suggesting a correlation between antimicrobial pressure and the emergence of resistance in pathogens (Allen et al., 2010; Bonnedahl, 2011). Although we consider the detection of multidrug resistant pathogens like Extended-spectrum beta-Lactamases producing Gram-negatives in wildlife as a new phenomenon, it could have been anticipated, as antimicrobial resistant bacteria other than intrinsically resistant soil organisms were already found in environmental samples apparently free from any antimicrobial pressure decades ago (Sato et al., 1978; Kanai et al., 1981; Hughes and Datta, 1983; Tsubokura et al., 1995).

While various bacterial species are important in terms of multiresistance and nosocomial infections in human and veterinary medicine, we consider the Gram-positive Methicillin resistant *Staphylococcus aureus* (MRSA) and Extended-spectrum beta-lactamases producing Gram-negative bacteria like *Escherichia coli* (ESBL-*E. coli*) as being key indicator pathogens to trace the evolution of multiresistant bacteria in the environment and wildlife. Both multiresistant organisms also made their way into livestock farming and companion animals (Smet et al., 2010b; Ewers et al., 2011; Wieler et al., 2011). Recent surveillance data on antimicrobial resistance among these organisms in human clinical settings display two major trends. According to the EARS-NET database[1] the prevalence of MRSA has remained on a high but stable level over the last years, whereas that of ESBL-*E. coli* has been on a continuous rise during the last decade. Although the majority of ESBLs are still reported from human clinical isolates (Bradford, 2001; Bonnet, 2004; Pitout, 2010), they are also increasingly recorded in community-acquired bacterial infections. This indicates that ESBL-*E. coli* have made their way out of the clinics, have been successfully transmitted and now persist in the community (Arpin et al., 2005; Pitout et al., 2005; Wieler et al., 2011).

To understand the dynamics of the dispersal of ESBL-*E. coli* into natural environments beyond human and domestic animal

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[1] http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/database/Pages/database.aspx

population, it is important to keep in mind the general *E. coli* population as well. *E. coli* is ubiquitous, and asymptomatically colonizes the gut of birds and mammals. Therefore, *E. coli* are found globally, not only in the gut but also in the environment (Wirth et al., 2006; Goldberg et al., 2008; Rwego et al., 2008). The intestinal population of *E. coli* in mammals and birds varies enormously between individuals even of the same species. This is why knowledge on the *E. coli* population of a single species is actually scarce and limited to single studies only, which do not represent the species as a whole (Schierack et al., 2008a,b; Leser and Molbak, 2009) It is however clear, that the use of antimicrobial compounds selects for resistant clones, with one mechanism being horizontal gene transfer between strains (LeClerc et al., 1996).

Although so far it is not clear how ESBL-*E. coli* make their way into the natural environment, they were seen to occur in the environment two decades after the first ESBL-*E. coli* outbreaks in human clinical settings (Kitzis et al., 1988; Bauernfeind et al., 1989; Costa et al., 2006). Simultaneously a community-onset of ESBL-*E. coli* has taken place and one might speculate whether environmental ESBL-*E. coli* are a spill-over form of environmental pollution from highly human influenced settings (Arpin et al., 2005; Pitout et al., 2005; Martinez, 2009a). Interestingly the first reports on ESBL-*E. coli* in wildlife date back shortly after their appearance in livestock farming which could also hint toward a manure driven spread of ESBL-*E. coli* into the environment (Kummerer, 2009). It seems unlikely that pathogens isolated from wildlife have acquired resistance through new parallel mutations in the respective genes. Horizontal transfer of resistance genes from clinical isolates or the intake of already resistant bacteria from human waste, sewage, and domesticated animal manure might be more probable (Kummerer, 2009; Martinez, 2009b).

Escherichia coli from wildlife may thus express a multiresistant phenotype, not due to the nearby use of antimicrobials or antimicrobials in subtherapeutic concentrations in natural environments, but because distant use had caused a multiresistant organism to evolve in the first place which subsequently spread to different ecological niches (O'Brien, 2002). The presence of commensal and pathogenic bacteria in fecal contaminations can be assumed to be a link between settings with regular or even constant antimicrobial pressure (livestock farming, aquaculture, human, and veterinary clinical settings) and the environment, resulting in a constant release of antibiotic-resistant human and animal bacteria into the environment through wastewater or manure (Martinez, 2009b). The detection of antimicrobial resistant bacteria in aquatic environments affected by human and animal wastewater and soil provides evidence for this hypothesis (Kummerer and Henninger, 2003). In this context the common use of antibiotics in aquaculture of fish is also of utmost importance due to possible direct influences on waterbirds (Baquero et al., 2008; Smith, 2008). As intestinal bacteria like *E. coli* can be easily disseminated in different ecosystems through water they are intensively used as indicator species for fecal pollution, but *S. aureus* is also regularly isolated from fecal samples. Therefore they could also be used to track the evolution of antimicrobial resistance into different ecosystems (Van Den Bogaard et al., 2000). Furthermore *E. coli* despite its commensal character is frequently implicated in animal and human infections that require the use of antibiotics which adds public health concerns to the

list of implications that arise from the spread of ESBL-*E. coli* into wildlife.

In contrast to studies on the appearance of ESBL-*E. coli* in humans and domesticated animals, their presence in wildlife has been addressed rarely. This review therefore summarizes currently available data on the presence of ESBL-*E. coli* in wildlife, concentrating on birds and rodents. It aims to bring about awareness about the urgent need to gather knowledge on the impact of ESBL-*E. coli* to the microbiota of wild animals and the consequences arising thereof for the environment and public health, acknowledging the zoonotic potential of *E. coli* and its abundance in nature (Allen et al., 2010; Bonnedahl, 2011).

BETA-LACTAM ANTIBIOTICS
The class of beta-lactam antibiotics is among the most important groups of antimicrobial agents in human and veterinary medicine. The chemical substances are in principal identical in both fields of clinical use. Besides the first widely used antimicrobial substance penicillin, other members of this family have gained a similar importance over the last decades, namely the first- to fourth-generation cephalosporins and the beta-lactamase-inhibitors. In the veterinary context the few studies that exist confirm that beta-lactam antimicrobials are the most commonly prescribed antimicrobials in small animals (DANMAP, 2007; SVARM, 2008). In livestock, a decrease in the use of beta-lactam antimicrobials could be observed over the last years (NORM/NORM-VET, 2010), basically due to restrictions in prescription. All beta-lactams interfere with the final stage of peptidoglycan synthesis through acting on penicillin-binding proteins, thereby preventing the bacterial cell wall from forming. The peptidoglycan constitutes a layer between the outer membrane and the cytoplasmic membrane which maintains the cell shape and protects the bacterium against osmotic forces. The most common resistance mechanism of Enterobacteriaceae spp. against beta-lactams is the inactivation of the drug by hydrolytic cleavage of the beta-lactam ring system (Greenwood, 2000).

BETA-LACTAMASES
More than 400 different beta-lactamase enzymes are currently known, sharing the same resistance mechanism but differing in their range of substrates and susceptibility against inhibitory substances[2]. Extended-spectrum beta-lactamases display an extended substrate spectrum, and this has directly influenced a global change in the epidemiology of beta-lactamases since the early 1990s in human medicine and since 2000 in veterinary medicine (Kong et al., 2010; Pitout, 2010; Smet et al., 2010b). The term extended-spectrum determines the ability of ESBLs to hydrolyze a broader spectrum of beta-lactam antimicrobials than the parent beta-lactamases they were originally derived from. While they are capable of inactivating beta-lactam antimicrobials containing an oxyimino-group such as oxyimino-cephalosporins (e.g., ceftazidime, cefotaxime) as well as oxyimino-monobactam (aztreonam), ESBLs are not active against cephamycins and carbapenems. They are usually inhibited by beta-lactamase-inhibitors like clavulanic acid and tazobactam, which marks a difference between

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[2]http://www.lahey.org/studies/

ESBL- and AmpC–beta-lactamases producing bacteria (Bradford, 2001). Several different classification schemes for bacterial beta-lactamases have been described, including the system devised by Bush et al. (1995) which is based on the activity of the beta-lactamases against different beta-lactam antimicrobials, and the currently most widely used Ambler system, which divides beta-lactamases into four classes (A, B, C, and D), based on their amino acid sequences (Ambler, 1980). The majority of ESBLs belong to Ambler class A and to the Bush group 2be. ESBLs have been found in a wide range of Gram-negative bacteria, but the vast majority of bacterial hosts belong to the family of Enterobacteriaceae, including *Klebsiella* spp., *E. coli*, *Salmonella enterica*, *Citrobacter* spp., and *Enterobacter* spp. (Bradford, 2001). Four enzyme families, namely TEM (Temoneira) -type beta-lactamases, SHV (Sulfhydryl variable) -type beta-lactamases, CTX (cefotaximase) -M-type beta-lactamases, and OXA (oxacillinase) -type beta-lactamases are currently regarded the most common ESBLs among Enterobacteriaceae spp. TEM-type beta-lactamases are derivatives of TEM-1, which was first demonstrated in 1965 in an *E. coli* isolate from a patient in Athens, Greece, named Temoneira, and of TEM-2 and consist of more than 150 different enzymes. While the majority of TEM beta-lactamases are ESBLs, TEM-1, TEM-2, and TEM-13 are only able to hydrolyse penicillin derivates and thus are not regarded as ESBLs (Livermore, 1995).

Similar to TEM-type enzymes the majority of SHV enzymes are ESBLs. All currently recognized SHV enzymes are derivatives of SHV-1 and SHV-2. Whereas SHV-1 merely confers resistance to broad-spectrum penicillins, SHV-2, which was first described in 1983 in a *Klebsiella ozaenae* strain isolated in Germany, is able to hydrolyse cefotaxime (Gupta, 2007). In contrast to TEM- and SHV-type beta-lactamases, most of the members of the OXA-type beta-lactamase family are not regarded as ESBLs because they do not hydrolyze third generation cephalosporins with the exception of OXA-10, OXA-2, and their derivatives[3]. However, distinct OXA-types (OXA-carbapenemases) play an important role in antimicrobial resistance, e.g., of *Acinetobacter baumannii* (Pfeifer et al., 2010).

Currently regarded as the most important ESBL enzyme family are the CTX-M-type beta-lactamases, named after their ability to hydrolyze cefotaxime. They are supposed to originate from beta-lactamases from *Kluyvera* spp. and currently comprise of more than 70 different CTX-M enzymes divided into five groups depending on their amino acid sequence (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25; Hawkey and Jones, 2009; Pitout, 2010; Naseer and Sundsfjord, 2011). AmpC–beta-lactamases confer resistance to most of the beta-lactam antimicrobials with the exception of methoxy-imino-cephalosporins (cefepime) and carbapenems, while they are not inactivated by beta-lactamase-inhibitors like clavulanic acid.

Within the last years the emergence of carbapenem-hydrolyzing beta-lactamases like NDM-1, KPC, and OXA has threatened the clinical utility of this antibiotic class (Pfeifer et al., 2010, 2011; Poirel et al., 2010; Walsh, 2010). Carbapenemases are beta-lactamases that are active not only against the oxyimino-cephalosporins and cephamycins but also capable of hydrolyzing carbapenems like imipenem or meropenem. These substances often display the "last line of defense" in the treatment of infections with multiresistant Gram-negative pathogens. This group of beta-lactamases is very diverse and can be found in three different β-lactamase classes (class A, B, and D). Detailed information on these enzymes is given in some excellent reviews (Walsh, 2010; Patel and Bonomo, 2011).

ESBLs IN HUMAN AND DOMESTIC ANIMALS

The first nosocomial outbreak of CTX-M-1 type ESBLs was recorded in an intensive care unit in a hospital in Paris, France (Kitzis et al., 1988). Shortly after that, Bauernfeind et al. (1989) reported on a clinical cefotaxime-resistant *E. coli* strain in Germany which produced a CTX-M-1 type beta-lactamase. In the following 10 years several studies reported about an explosive dissemination of ESBLs in human clinical settings worldwide (Bernard et al., 1992; Gniadkowski et al., 1998; Radice et al., 2002; Canton and Coque, 2006). Several review articles provide detailed insight into the occurrence and molecular epidemiology of ESBL producing Enterobacteriaceae in humans and animals (Bradford, 2001; Bonnet, 2004; Canton and Coque, 2006; Livermore et al., 2007; Cantón et al., 2008; Oteo et al., 2010; Pfeifer et al., 2010; Pitout, 2010; Wieler et al., 2011).

Since about 2000, the CTX-M enzymes have formed a rapidly growing family of ESBLs in human clinical and community settings (Bonnet, 2004; Pitout and Laupland, 2008; Mshana et al., 2009), whereas the prevalence of classical ESBL enzymes like TEM or SHV is decreasing (Livermore et al., 2007). With the beginning of the twenty-first century *E. coli* producing CTX-M-15 have emerged and disseminated worldwide as an important cause of both nosocomial and community-onset urinary tract and bloodstream infections in humans (Coque et al., 2008; Hunter et al., 2010; Oteo et al., 2010; Pitout, 2010). A number of molecular epidemiological studies revealed that the sudden worldwide increase of CTX-M-15-producing *E. coli* has been largely influenced by the spread of one single clonal group of strains, namely B2:O25b:H4-ST131-CTX-M-15, across different continents (Nicolas-Chanoine et al., 2008; Rogers et al., 2011). The recent emergence of yet another clonal group, ABD-O1:H6-ST648-CTX-M-15, envisions the potential of just a limited number of clones to spread globally (Doi et al., 2010; Zong and Yu, 2010; Van Der Bij et al., 2011; Wieler et al., 2011). Unraveling the microevolution of strains of these clones in habitats and ecological niches others than human and veterinary clinics offers the chance to understand what leads to persistence of ESBL-*E. coli* in surroundings lacking selective antibiotic pressure.

In the field of veterinary medicine an SHV-12-type beta-lactamase producing *E. coli* was the first clinical ESBL producing bacteria isolated from a dog with recurrent urinary tract infection in Spain in 1998 (Teshager et al., 2000). This was followed by the detection of ESBL producing *E. coli* (mostly TEM and SHV) in dogs from Italy, and Portugal (Feria et al., 2002; Carattoli et al., 2005). Very recently several studies also reported companion animals as hosts for ESBL-*E. coli* harboring CTX-M enzymes, leading to the assumption that CTX-M-type enzymes will dominate the situation in veterinary medicine in the future as well (Vo et al.,

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[3]http://www.lahey.org/studies/

2007; Carattoli, 2008; O'Keefe et al., 2010; Smet et al., 2010b). This is exemplified by the emergence of the clonally related group of *E. coli* B2-O25b:H4-ST131-CTX-M-15 in the field of companion animals, as well (Pomba et al., 2009; Ewers et al., 2010, 2011; Biohaz, 2011; Wieler et al., 2011).

Extended-Spectrum beta-lactamases mostly of the TEM, CTX-M, and SHV-type have been frequently demonstrated in the microbiota of food-producing animals which has nicely been reviewed by Smet et al. (2010b). Within the last decade, the number of publications reporting ESBL-*E. coli* isolated from food-producing animals has increased drastically. Noticeably, most ESBL enzymes identified in *E. coli* from livestock are likewise present in bacteria from humans (Smet et al., 2010b).

ESBLs IN WILDLIFE

A HISTORICAL PERSPECTIVE

The first reports on the presence of resistance determinants in *E. coli* from human and animal populations lacking selective antimicrobial pressure date back to the 1960s (Mare, 1968). Antimicrobial resistant *E. coli* isolates originating from wildlife species were reported for the first time at the beginning of the 1980s from Japanese wild birds (Sato et al., 1978; Kanai et al., 1981; Tsubokura et al., 1995) and 5 years later in South African baboons feeding on human refuse (Rolland et al., 1985; Routman et al., 1985). With the new millenium the number of studies describing the occurrence of antimicrobial resistant *E. coli* in wildlife increased significantly (Gilliver et al., 1999; Souza et al., 1999; Sherley et al., 2000; Fallacara et al., 2001; Livermore et al., 2001; Osterblad et al., 2001; Swiecicka et al., 2003; Cole et al., 2005; Lillehaug et al., 2005; Middleton and Ambrose, 2005; Sayah et al., 2005; Skurnik et al., 2006; Dolejska et al., 2007; Literak et al., 2007; Carattoli, 2008; Gionechetti et al., 2008; Ewers et al., 2009; Guenther et al., 2010c).

However, the detection of ESBL-*E. coli* of wildlife origin dates back to 2006 only (Costa et al., 2006). Since then several reports followed (Costa et al., 2008; Poeta et al., 2008, 2009; Bonnedahl et al., 2009, 2010; Dolejska et al., 2009; Literak et al., 2009a,b, 2010; Guenther et al., 2010a,b; Hernandez et al., 2010; Pinto et al., 2010; Radhouani et al., 2010; Simoes et al., 2010; Smet et al., 2010b; Garmyn et al., 2011; Ho et al., 2011; Silva et al., 2011; Sousa et al., 2011; Wallensten et al., 2011).

A GEOGRAPHICAL PERSPECTIVE

Although ESBL-*E. coli* isolates of wildlife origin have only been reported from Europe (Costa et al., 2008; Poeta et al., 2008, 2009; Bonnedahl et al., 2009, 2010; Dolejska et al., 2009; Literak et al., 2009b, 2010; Guenther et al., 2010a,b; Pinto et al., 2010; Radhouani et al., 2010; Simoes et al., 2010; Garmyn et al., 2011; Silva et al., 2011; Sousa et al., 2011; Wallensten et al., 2011), Africa (Literak et al., 2009a), and Asia (Hernandez et al., 2010; Ho et al., 2011) so far, their absence in the Americas, Antarctica, and Australia might simply reflect the different number of studies performed in these continents. As multiresistant *E. coli* have already been reported from the latter continents (Souza et al., 1999; Sherley et al., 2000; Fallacara et al., 2001; Cole et al., 2005; Middleton and Ambrose, 2005; Sayah et al., 2005; Kozak et al., 2009; Silva et al., 2009) one could anticipate ESBL-*E. coli* of wildlife origin to be present as well. Nevertheless data from one continent or region may not act as a

suitable baseline for another, and may not correlate with the level of antibiotic use in the regions involved. Besides simple geographical effects like the continent of origin it seems more appropriate to reconsider the type of region where the isolates originate from. Parameters which have been assumed as important criteria include the natural preservation state, livestock, and human density or the remoteness of an area (Allen et al., 2010). The level of resistant bacteria observed in wild animals seems to correlate well with the degree of association with human activity (Skurnik et al., 2006; Allen et al., 2010). Nevertheless, several studies report the occurrence of ESBL-*E. coli* in remote places or preservation areas as well (Hernandez et al., 2010; Pinto et al., 2010) underlining the complexity of the spread of antimicrobial resistance in wild animals. These findings suggest on the one hand an influence of migratory behavior of wild birds for instance into remote areas or on the other hand the omnipresence of human influence in various ecological niches of the planet basically via human feces. Most studies on ESBL-*E. coli* in wildlife originate from Central Europe, an area with high livestock and human density and an assumable frequent interaction of wildlife with human influenced habitats of any kind like livestock farms, landfills, sewage systems, or wastewater treatment facilities, resulting in a higher risk for wildlife acquiring antibiotic-resistant bacteria (Allen et al., 2010). It has previously been shown that gulls shared strains of *E. coli* with isolates cultured from landfills and wastewater treatment plants (Nelson et al., 2008). This underlines the possibility of bacterial exchange between human sewage and birds.

As summarized in **Table 1** the detection rates of ESBL-*E. coli* in different geographical areas ranged from 0.5% in birds of the remote Azores islands in the Atlantic Ocean (Silva et al., 2011) to 32% for birds of the Iberean peninsula (Simoes et al., 2010). However, one should certainly keep in mind differences with regards to host species, sampling schemes, and geographic regions and the limitations that arise from this when interpreting these data. Nevertheless, for Central Europe the number of studies performed is relatively high, and there does not seem to be a difference in the detection rates between agriculturally used lands or urban environments compared to natural preserve areas, since in both types of areas detection rates higher than 20% have been observed (**Table 1**). Only in remote areas like the Azores or the Kamchatka peninsula the rates seem to be lower with approximately 1% ESBL-*E. coli* (Hernandez et al., 2010; Silva et al., 2011), suggesting a possible dilution effect of the pollution of wild animals with ESBL-*E. coli*. Nevertheless our own data from birds of prey from the Mongolian Gobi-Desert, an area among the ones with the lowest human density, revealed ESBL rates which were comparable with the situation in Central Europe (Guenther et al., 2010c).

A HOST SPECIES PERSPECTIVE

General information about the microbiota of wild living birds and rodents is scarce and restricted to single species as hosts of certain pathogens. *E. coli* is a common gastrointestinal but very versatile bacterium, and can be grouped into non-pathogenic (commensal) and pathogenic strains; the latter cause intestinal or extraintestinal diseases in humans and animals (Johnson and Russo, 2002; Wirth et al., 2006). The ubiquitous occurrence of *E. coli* is based on its asymptomatical colonization of the gut of birds and mammals

Table 1 | Presence of extended-spectrum beta-lactamases producing *E. coli* in wildlife in chronological order according to the date of publication.

Reference	Animal species	No. of ESBL producing isolates per total no. (%) of isolates investigated	Detected ESBL types (% in relation to total no. of ESBL)	Country	Year of isolation	MLST (no. of isolates)
Costa et al. (2006)	Bird of prey, Deer, Fox, Owl (all unspecified)	9/56 (16.1)	bla_{TEM-52} (33), $bla_{TEM-52} + bla_{CTX-M-14}$ (11), $bla_{CTX-M-14} + bla_{TEM-1}$ (22), $bla_{CTX-M-1} + bla_{TEM-1}$ (11), bla_{SHV-12} (11), $bla_{CTX-M-14}$ (11)	Portugal	2003–2004	Not specified
Poeta et al. (2008)	Seagulls (*Larus* sp.)	11/57 (19.3)	bla_{TEM-52} (72.7), $bla_{CTX-M-1}$ (9.1), $bla_{CTX-M-14a}$ (9.1), $bla_{CTX-M-32}$ (9.1)	Portugal	2007	Not specified
Dolejska et al. (2009)	Black headed gull (*C. ridibundus*)	7/213 (3.2)	$bla_{CTX-M-1}$ (14.2), $bla_{CTX-M-15}$ (28.6), bla_{SHV-2} (14.2), bla_{SHV-12} (28.6), unknown (14.2)	Czech Republic	2005	Not specified
Bonnedahl et al. (2009)	Yellow legged gull (*L. michahellis*)	16/180 (8.8)	$bla_{CTX-M-1}$ (43.8), $bla_{CTX-M-1} + bla_{TEM-1}$ (6.6), $bla_{CTX-M-15} + bla_{TEM-1}$ (6.6), bla_{TEM-1} (31.3), $bla_{SHV} + bla_{TEM-1}$ (12.5)	France	2008	ST1199, ST533, ST1140 (2), ST156, ST90, ST1142, ST681 (2), ST1134, ST1143 (2), ST1135, ST1144, ST746, ST351
Poeta et al. (2009)	Wild boar (*S. scrofa*)	8/77 (10.3)	$bla_{CTX-M-1}$ (75), $bla_{CTX-M-1} + bla_{TEM-1}$ (25)	Portugal	2005–2007	Not specified
Literak et al. (2009a)	Brown rat (*R. rattus*)	1/37 (2.3)	$bla_{CTX-M-15}$ (100)	Senegal	2007	Not specified
Hernandez et al. (2010)	Glaucous winged gull (*L. glaucescens*)	4/532 (0.8)	$bla_{CTX-M-14}$ (50), $bla_{CTX-M-15}$ (50)	Russia	2007	ST131, ST609 (2), ST746
Bonnedahl et al. (2010)	Black headed gull (*C. ridibundus*)	3/83 (3.6)	$bla_{CTX-M-14}$ (66), $bla_{CTX-M-15}$ (33)	Sweden	2008	ST1646, ST1340, ST1647
Literak et al. (2010)	Mallard duck (*A. platyrhynchos*), Herring gull (*L. argentatus*)	9/83 (10.8)	$bla_{CTX-M-1}$ (66), $bla_{CTX-M-9} + bla_{TEM-1b}$ (11), $bla_{CTX-M-15} + bla_{OXA-1}$ (11), bla_{SHV-12} (11)	Poland	2008–2009	Not specified
Pinto et al. (2010)	Buzzard (*B. buteo*), Barn owl (*T. alba*), Tawny owl (*S. aluco*), Booted eagle (*A. pennata*), Montagu's harrier (*C. pygargus*), Black kite (*M. migrans*), Black vulture (*C. atratus*), Bonelli's eagle (*A. fasciata*), Eurasian eagle owl (*B. bubo*), Raven (*C. corax*)	32/119 (26.9)	$bla_{CTX-M-1}$ (40.5), $bla_{CTX-M-1} + bla_{TEM-1}$ (43.8), $bla_{CTX-M-1} + bla_{TEM-20}$ (3.1), bla_{SHV-5} (3.1), $bla_{SHV-5} + bla_{TEM-1}$ (6.3), $bla_{SHV-5} + bla_{TEM-20}$ (3.3)	Portugal	2008	Not specified
Radhouani et al. (2010)	Buzzards (*B. buteo*)	5/33 (15.2)	$bla_{CTX-M-32} + bla_{TEM-1}$ (70), $bla_{CTX-M-1} + bla_{TEM-1}$ (30)	Portugal	2007–2008	Not specified

(Continued)

Table 1 | Continued

Reference	Animal species	No. of ESBL producing isolates per total no. (%) of isolates investigated	Detected ESBL types (% in relation to total no. of ESBL)	Country	Year of isolation	MLST (no. of isolates)
Simoes et al. (2010)	Seagulls (*Larus* sp.)	45/139 (32)	$bla_{CTX-M-1}$ (18), $bla_{CTX-M-9}$ (9), $bla_{CTX-M-15}$ (39), $bla_{CTX-M-32}$ (34)	Portugal	2007–2008	ST1284 (4), ST131 (4), ST224 (3), ST453, ST86, ST205, ST359, ST165, ST69, ST1152, ST405, ST559, ST1163, ST10, ST58, ST156, ST155, ST297, ST43, ST58, ST156
Wallensten et al. (2011)	Seagulls (*Larus* sp.)	18/194 (9.2)	$bla_{CTX-M-1} + bla_{TEM-1}$ (50), $bla_{CTX-M-1} + bla_{TEM-1}$ (38.8), $bla_{CTX-M-14} + bla_{TEM-1}$ (5.5), bla_{SHV-12} (5.5)	Sweden	2010	Not specified
Silva et al. (2011)	Black cap (*S. atricapilla*)	1/220 (0.45)	$bla_{CTX-M-14} + bla_{SHV-12}$ (100)	Azores/ Portugal	2006–2010	Not specified
Sousa et al. (2011)	Gilthead sea bream (*S. aurata*)	5/118 (4.2)	bla_{TEM-52} (40), bla_{SHV-12} (60)	Atlantic ocean/ Portugal	2007	Not specified
Ho et al. (2011)	Rodents (unspecified)	19/456 (4.2)	$bla_{CTX-M-9}$ (42.6), $bla_{CTX-M-1}$ (47.4)	China	2008–2010	Not specified
Guenther et al. (2010b)	Norway rat (*R. norvegicus*)	1/220 (0.5)	$bla_{CTX-M-9}$	Germany	2009	ST131
Guenther et al. (2010a)	Eurasian blackbird (*T. merula*), White fronted goose (*A. albifrons*), Rock pigeon (*C. livia*)	4/172 (2.3)	$bla_{CTX-M-15}$ (100)	Germany	2009	ST648
Literak et al. (2009b)	Wild boars (*S. scrofa*)	5/293(2)	$bla_{CTX-M-1} + bla_{TEM-1}$ (20), $bla_{CTX-M-1}$ (60), $bla_{TEM-52b}$ (20)	Czech Republic	2009	Not specified
Garmyn et al. (2011)	Wild geese (*B. canadensis*, *A. anser*)	2/396 (0.5)	bla_{TEM-52} (50), bla_{SHV-12} (50)	Belgium	2010	ST1079, ST1844

and in turn the resulting spread into the environment (Rwego et al., 2008; Schierack et al., 2008a). The degree of colonization varies a lot between different bird species (Gordon and Cowling, 2003) and the same has been shown for rodents and small mammals (Swiecicka et al., 2003; Guenther et al., 2010c). *E. coli* is most likely to be isolated from omnivore birds and mammals (Gordon and Cowling, 2003). More than 30 wild animal species have been found shedding ESBL-*E. coli* and most of them were birds or rodents (**Table 1**). The occurrence of ESBL-*E. coli* is therefore clearly influenced by the host spectrum of *E. coli* and furthermore by the degree of synanthropic behavior shown by the host animal species. In other words, animals living in urbanized areas are more likely to carry *E. coli* than animals living in remote areas (Allen et al., 2010; Bonnedahl, 2011).

Other basic questions concerning the occurrence of ESBL-*E. coli* in fecal samples are still unsolved. Future studies should therefore address the nature of the abundance of these multiresistant strains in feces to clarify if they are just shedded in short terms, present transient, or long term colonizations of

the gut of the animals asymptomatically or even are persistent infections.

When reviewing the current literature it appears that wild birds could be the main wildlife hosts for ESBL-*E. coli*. This impression is created because most of the studies were carried out on wild birds; however, taking into account studies involving other wildlife animals such as deer, small ruminants, small and large predators, lagomorphs, reptiles, and amphibians (Costa et al., 2006; Literak et al., 2009b) an insignificant number of ESBL-*E. coli* was observed.

Due to their diversity in ecological niches, and their ease in picking up human and environmental bacteria, wild birds might act as mirrors of human activities. Within the heterogeneous class of birds two groups seem to be in the focus of ESBL carriage in wildlife: waterfowl/water related species (Poeta et al., 2008; Bonnedahl et al., 2009, 2010; Dolejska et al., 2009; Guenther et al., 2010b; Hernandez et al., 2010; Literak et al., 2010; Simoes et al., 2010; Garmyn et al., 2011; Wallensten et al., 2011) and birds of prey (Costa et al., 2006; Pinto et al., 2010; Radhouani et al., 2010; detailed species information is given in **Table 1**). Other groups

of birds like passerines (Guenther et al., 2010a; Silva et al., 2011) seem to carry ESBL-*E. coli* less often or sometimes not at all (Silva et al., 2010). This finding might be influenced by differences in the composition of the microbiota and the harboring of *E. coli* within these diverse avian species. In a recent study we were able to show that if *E. coli* could be isolated from different bird species, multiresistant *E. coli* clones originated from birds of prey or waterfowl (Guenther et al., 2010d). Other avian hosts reported were Owls (Costa et al., 2006; Pinto et al., 2010) and Ravens (Pinto et al., 2010), birds which display a feeding behavior comparable to birds of prey.

While the dominance of waterfowl within the avian host spectrum of ESBL-*E. coli* can be explained by fecal pollution of water by human or livestock sources, the transmission scenarios for the other main group – birds of prey – seem to be more complex. As birds of prey are on top of the food chain they could accumulate ESBL-*E. coli* from their typical prey, like mice and shrews. Due to their synantropic behavior, they are presumably more often in contact with humans or livestock. Indeed for mice it has been shown that proximity to livestock farming increases the carriage rates of multiresistant *E. coli* (Kozak et al., 2009). Although rodents have earlier been in the focus of research on ESBL in wildlife (Gilliver et al., 1999; Kozak et al., 2009; Literak et al., 2009b; Guenther et al., 2010c), to the best of our knowledge ESBL-*E. coli* have not yet been detected in rodents with the exception of urban rats (Guenther et al., 2010b; Ho et al., 2011).

Our own data from Germany revealed a very low abundance for multiresistant *E. coli* in rodent and shrews, indicating that other groups of prey and transmission routes between the human influenced ecosphere and birds of prey might be possible (Guenther et al., 2010c). Furthermore, many of the bird species which have been tested positive for ESBL-*E. coli* carriage display migration behavior which provides a possible mechanism for the establishment of new endemic foci over great distances from where a multiresistant microorganism was first acquired (Bonnedahl, 2011).

As mentioned above another important host of ESBL-*E. coli* seems to be a group of rodents namely Norway rats and Black rats with reports on ESBL producing isolates from different continents (Literak et al., 2009a; Guenther et al., 2010b; Ho et al., 2011). This synantropic species can easily pick up human waste and often interacts with human feces in the sewage system in urban environments and can therefore easily acquire multiresistant bacteria. Interestingly wild boars have also been reported as host s of ESBL-*E. coli* in Central Europe, which might reflect their omnivorous feeding behavior (Literak et al., 2009b; Poeta et al., 2009). Other mammals found to be positive hosts of ESBL producing bacteria were deer and foxes (Costa et al., 2006). Very recently there has been a report on a marine fish, the Gilthead Sea bream (Sousa et al., 2011) as a carrier of ESBL-*E. coli*, indicating a dissemination of ESBL-*E. coli* into the Atlantic ocean.

A PERSPECTIVE ON ESBL ENZYMES

In parallel to the current situation in human and veterinary medicine the type of extended-spectrum beta-lactamases found in wild animals are clearly dominated by the bla_{CTX-M} gene-family. With the exception of one study (Sousa et al., 2011) all wildlife studies

identified the bla_{CTX-M} genes as the main ESBL enzyme. In 35% of the studies different SHV enzymes were additionally detected. As shown in **Table 1**, most of the studies reported $bla_{CTX-M-1}$, followed by $bla_{CTX-M-15}$, $bla_{CTX-M-14}$, $bla_{CTX-M-32}$, and $bla_{CTX-M-9}$. Only occasionally $bla_{CTX-M-2}$, $bla_{CTX-M-13}$, $bla_{CTX-M-55}$, and $bla_{CTX-M-65}$ were detected (**Table 1**). Besides the bla_{CTX-M}-type family only bla_{SHV-12} and bla_{TEM-52} were also often detected. Other less prevalent ESBL genes were bla_{OXA-1}, bla_{SHV-5}, and bla_{TEM-20}. The spectrum of the different enzyme types found in wild animals is very narrow compared to clinical isolates of human and veterinary origin (Pitout, 2010; Smet et al., 2010b). While the reason for this is unknown, we offer the following hypotheses: these findings might simply reflect the small number of studies performed on wildlife so far, or they could indicate that certain types of beta-lactamases are more successful in the environment, for example due to co-selection of other non-resistance genes accompanied by these beta-lactamases. Another explanation could be that the types of ESBLs found in wild animals simply reflect the ones that are most prevalent in human and veterinary clinics and in livestock farming, such as $bla_{CTX-M-1}$ or $bla_{CTX-M-15}$ (Pitout, 2010; Smet et al., 2010b). This could lead to the assumption that the situation we are observing in wild animals is just presenting spill-over effects from clinics and livestock farming. If this was true, future studies presumably should find a rise in those pandemic CTX-M-15 types, exemplified by the clonal *E. coli* lineages of ST131 and ST648. Several studies observed a similarity in the overall resistance profiles of wild animal isolates with human or veterinarian clinical isolates which supports this hypothesis (Costa et al., 2008; Literak et al., 2009b; Guenther et al., 2010d). The most prevalent non-ESBL resistant phenotype in wildlife *E. coli* is resistance to streptomycin, ampicillin, and tetracycline. This pattern is also very common in human and livestock populations in Europe (Van Den Bogaard et al., 2000; Guerra et al., 2003). As mentioned above the types of ESBL genes are basically the same in human, livestock, and wildlife, which strengthens the hypothesis that wildlife isolates resemble those found in animal and human patients. However, as the dissemination of ESBL genes is highly driven by horizontal gene transfer through plasmids, the occurrence of identical ESBL genes could also be based on the spread of ESBL-plasmids which are randomly distributed in the environment. In summary, unraveling the basis of the ESBL-*E. coli* spill-over into wildlife needs to include both a characterization of the clonal nature of bacterial strains isolated from different hosts as well as an accurate identification of the ESBL genes and their episomal or chromosomal localization.

A ZOONOTIC POTENTIAL PERSPECTIVE

Regarding the basic question of the zoonotic potential of *E. coli* it is useful to address the population genetics of this bacterial species. Besides comparative whole genome analysis of bacteria, this can also be done by other approaches like Multi-locus sequence typing (MLST)[4]. Although it is based on a small set of marker genes only, this method seems to reflect the microevolution of the *E. coli* core genome. In general, MLST analysis revealed the existence of strains belonging to identical sequence types (STs) and

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[4]www.mlst.net

being isolated from different hosts rather being the rule than the exception. This indicates a common phylogeny and therefore a zoonotic potential for most strains analyzed so far (Wirth et al., 2006). Indeed, several research groups found clusters of *E. coli* causing systemic infections in birds, and urinary tract infection and neonatal meningitis in humans, which are genetically so similar, that a zoonotic potential is foreseen (Johnson and Russo, 2002; Ewers et al., 2007; Moulin-Schouleur et al., 2007). So far the number of ESBL-*E. coli* from wildlife in that global data base is rather limited. However, if the same clusters of *E. coli* can cause disease in humans and domesticated birds, their transmission scenarios become important and such routes of transfer indeed are a plausible transfer mechanism of ESBL-*E. coli* from humans to wild birds and vice versa.

To answer the question about the similarity of human clinical, livestock, companion animal, and wildlife ESBL-*E. coli* isolates we need to gain insight into the clonal relatedness of isolates from all these groups. Initial MLST paired with pulsed field gel electrophoresis (PFGE) is an ideal tool to reveal clonal relatedness or even clonal identity of epidemiologically unrelated isolates. This attempt has been put forward by a small number of studies on wild birds, all clearly pointing out that similar STs or clonal groups are present in humans, domestic animals, and wild birds (Bonnedahl et al., 2009, 2010; Guenther et al., 2010a; Hernandez et al., 2010; Simoes et al., 2010). Overall up to 35 different STs have been detected in wild avian ESBL-*E. coli* (**Table 1**; **Figure 1**). Although some of the "avian" STs appeared twice in different wildlife studies like ST746 (Bonnedahl et al., 2009; Hernandez et al., 2010) and have not been detected in human clinical samples yet, the majority of the STs found in avian ESBL-*E. coli*, such as ST131, ST10, ST90, ST648, or ST69, are also present in human clinical isolates.

Figure 1 shows a minimum spanning tree (MSTree) displaying human, domestic animal, and wildlife ESBL–STs based on data of the MLST database[5] as of October 2011 and the current literature on human and animal ESBL-*E. coli* isolates providing MLST data (Minarini et al., 2007; Yumuk et al., 2008; Blanco et al., 2009; Hrabak et al., 2009; Naseer et al., 2009; Oteo et al., 2009; Sidjabat et al., 2009; Suzuki et al., 2009; Valverde et al., 2009; Coelho et al., 2010; Doi et al., 2010; Peirano et al., 2010; Smet et al., 2010a; Zong and Yu, 2010; Ben Slama et al., 2011; Djamdjian et al., 2011; Leverstein-Van Hall et al., 2011; Mshana et al., 2011; Van Der Bij et al., 2011; Woerther et al., 2011). In the MSTree each circle represents a ST and the size of the circle is proportional to the number of ESBL-*E. coli* isolates belonging to this ST. Here is becomes remarkably clear that ESBL-*E. coli* of wildlife, domestic animal, and human origin share identical STs suggesting an interspecies transmission of phylogenetically related multiresistant strains. This hypothesis is further strengthened by the detection of the worldwide emerging clonal group of *E. coli* specified as B2-O25b:H4-ST131 in human (Nicolas-Chanoine et al., 2008), veterinary clinical settings (Ewers et al., 2010), and in a Glaucous winged gull in Kamchatka (Hernandez et al., 2010).

In another study (Bonnedahl et al., 2010) in south Sweden ESBL-*E. coli* of several new STs were detected, including ST1646
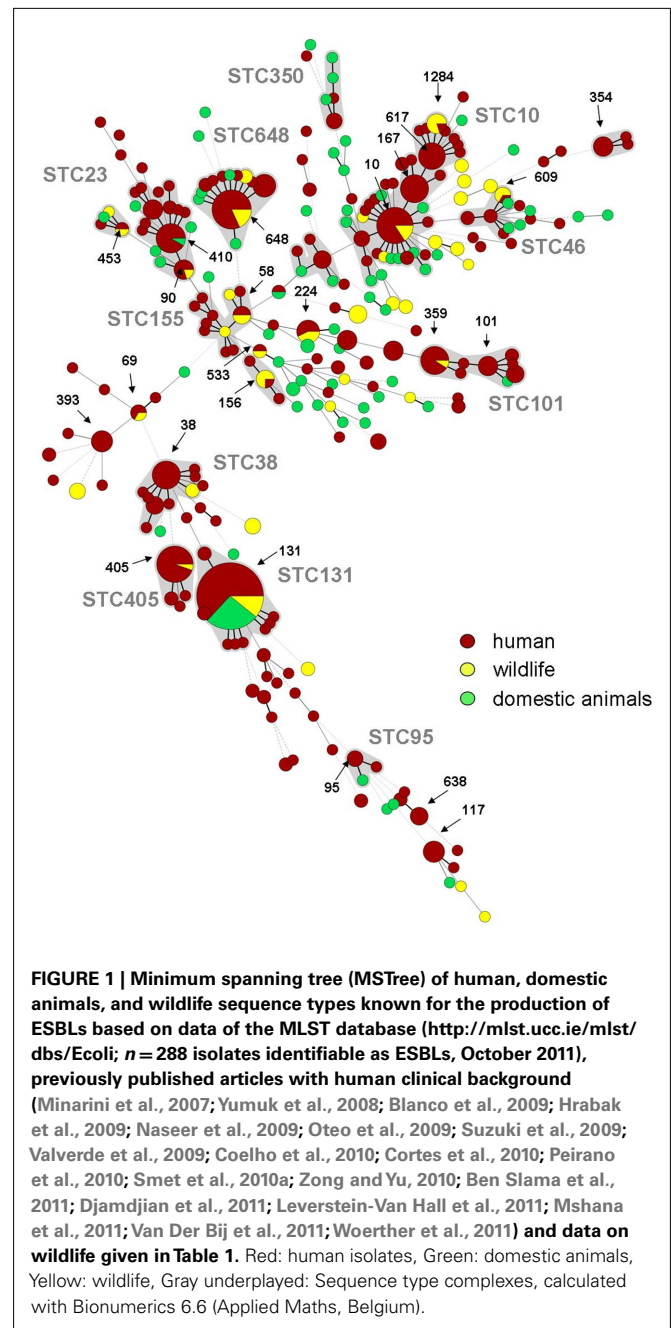
[5]http://mlst.ucc.ie/mlst/dbs/Ecoli

FIGURE 1 | Minimum spanning tree (MSTree) of human, domestic animals, and wildlife sequence types known for the production of ESBLs based on data of the MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli; *n* **= 288 isolates identifiable as ESBLs, October 2011), previously published articles with human clinical background (Minarini et al., 2007; Yumuk et al., 2008; Blanco et al., 2009; Hrabak et al., 2009; Naseer et al., 2009; Oteo et al., 2009; Suzuki et al., 2009; Valverde et al., 2009; Coelho et al., 2010; Cortes et al., 2010; Peirano et al., 2010; Smet et al., 2010a; Zong and Yu, 2010; Ben Slama et al., 2011; Djamdjian et al., 2011; Leverstein-Van Hall et al., 2011; Mshana et al., 2011; Van Der Bij et al., 2011; Woerther et al., 2011) and data on wildlife given in Table 1.** Red: human isolates, Green: domestic animals, Yellow: wildlife, Gray underplayed: Sequence type complexes, calculated with Bionumerics 6.6 (Applied Maths, Belgium).

which is closely related to ST648 previously also found in wild birds in Germany (Guenther et al., 2010a) and in humans in Africa, Asia, and the United States (Doi et al., 2010; Zong and Yu, 2010). Interestingly, ST648 has been detected as one of the STs associated with the carriage of the newly emerging carbapenemase NDM-1 which underlines unforeseeable consequences of the entry of certain multiresistant clones into wild bird populations (Mushtaq et al., 2011).

As mentioned above there are currently three studies that provide evidence for the frequent occurrence of ESBL-*E. coli* in rats (Literak et al., 2009a; Guenther et al., 2010b; Ho et al., 2011). However, comparative data on the clonal relatedness of these isolates

and those of human or domestic animals as assessed by MLST or PFGE is limited to a single study only (Guenther et al., 2010b). Here an ESBL-*E. coli* from a rat belonging to the pandemic clone B2-O25b:H4-ST131 was detected, which might point toward a direct transmission from human feces to the rat in an urban sewage system.

CONCLUSION

The current data on ESBL-*E. coli* in wild animals reveals that carriage of these multiresistant strains is widespread in at least some wild populations like waterfowl, birds of prey, and rodents, even though these have never been exposed continuously to antibiotics. This clearly undermines the presumption that resistance will decline with the absence of antibiotic treatment alone. It underlines the very complex nature of the spread of antimicrobial resistance which has been also already pointed out for non-beta-lactam resistance in human populations in remote areas in South America (Pallecchi et al., 2007; Bartoloni et al., 2009).

The origins of resistance and the selection mechanisms responsible for maintaining high prevalence of resistance are largely unknown and therefore need to be addressed more soundly (Gilliver et al., 1999). The common occurrence of ESBL-*E. coli* in wildlife, especially in avian hosts, has several implications. Firstly, wildlife has the potential to serve as an environmental reservoir and melting pot of bacterial resistance. Secondly by taking into account the zoonotic potential of *E. coli* and the concomitant observation that ESBL-*E. coli* of wildlife origin are basically the same than the ones found in clinical isolates they additionally have the potential to re-infect human populations. Bird's feces are omnipresent in urban and rural settings and smear infections of humans by avian droppings should not be underestimated. Many bird species, including those that were already identified as carriers of ESBL-*E. coli*, display considerable mobility, often involving the crossing of continents. In the same way the phenomenon of bird migration creates the potential for the establishment of new endemic foci of disease along their routes like it has been seen for the West Nile Virus in the USA (Reed et al., 2003), antimicrobial

resistant bacteria might also be carried over long distances by avian hosts. Bird migration could therefore contribute to the dissemination of resistance over the globe as has previously been observed for human travelers (Peirano et al., 2011). One might think that this is of minor impact compared to human travel but we have to keep in mind that in contrast to the human population there is no sewage system for bird feces, and droppings are therefore directly exposed to the environment as well as to human and animal population.

Avian mobility and spread of ESBL-*E. coli* might also have unpredictable consequences through possible interactions of these birds with environmental bacterial ecosystems in remote areas reached mainly by migrating birds. We have to keep in mind that the role of antimicrobial substances or their ancestors in natural ecosystems like soil differs considerably from their antinfective function in clinical settings. This in turn means that the crosstalk of bacterial communities in fragile ecosystems could be highly influenced by the entry of multiresistant bacteria (Allen et al., 2010).

Future studies should address whether ESBL-*E. coli* can persist in the environment or circulate in animals for long periods and may thus be disseminated by wildlife and other vectors. The pandemic spread of certain ESBL-*E. coli* lineages into the environment highlights the complexity of dissemination of antimicrobial drug resistance. As previously suggested, thorough spatial and temporal studies of antimicrobial drug resistance in different natural habitats are warranted (Gilliver et al., 1999; Hernandez et al., 2010) to fully understand the importance of wildlife as a source of antimicrobial resistance.

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