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Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates collected from diseased food-producing animals in the GE*RM*-Vet monitoring program 2008–2014

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ABSTRACT

The aim of this study was to identify extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* collected from diseased food-producing animals in Germany. A total of 6849 *E. coli* isolates, collected from diseased cattle, pigs and poultry in the German national monitoring program GE*RM*-Vet (2008–2014), were characterized by antimicrobial susceptibility testing and screened for the ESBL phenotype. ESBL genes were identified by PCR and sequencing. The isolates were further characterized by PCR-based phylotyping. The 419/6849 (6.1%) ESBL-producers identified included 324/2896 (11.2%) isolates from cattle, 75/1562 (4.8%) from pigs and 20/2391 (0.8%) from poultry. The ESBL genes detected were: $bla_{CTX\text{-}M\text{-}1}$ (69.9%), $bla_{CTX\text{-}M\text{-}15}$ (13.6%), $bla_{CTX\text{-}M\text{-}14}$ (11.7%), $bla_{TEM\text{-}52}$ (1.9%), $bla_{SHV\text{-}12}$ (1.4%), $bla_{CTX\text{-}M\text{-}3}$ (1.0%), and $bla_{CTX\text{-}M\text{-}2}$ (0.5%). The phylogroup A was the dominant phylogroup (57.0%) followed by phylogroups D (23.4%), B1 (17.9%), and B2 (1.7%). Bovine isolates belonged predominantly to the phylogroups A and D, whereas the porcine and avian isolates mainly belonged to A and B1. The majority of the ESBL-producing isolates found in each phylogroup were from animals suffering from gastrointestinal infections. In 399/419 isolates (95.2%), additional resistance to non-β-lactam antibiotics was seen. Multidrug-resistance [resistance to aminoglycosides, fluoro(quinolones), sulphonamides, tetracyclines, and trimethoprim] was seen in 369/419 (88.1%) isolates, which may facilitate the co-selection of ESBL genes, when located on the same mobile genetic element as the others resistance genes, and may compromise the therapeutic options.

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1. Introduction

Food-producing animals are an important reservoir of antimicrobial-resistant zoonotic bacterial pathogens. For the control of bacterial infections, β-lactam antibiotics play an important role in human and veterinary medicine. In 2012, antimicrobial agents of the class of β-lactam antibiotics (penicillins) were the second most sold agents to veterinarians in Germany (Wallmann and Römer, 2012), but the latest data from 2014 identified β-lactams as the most frequently sold antimicrobial agents in veterinary medicine (450 tons), followed by tetracyclines (342 t), and sulfonamides (121 t) (https://www.bvl.bund.de/DE/08_PresseInfothek/01_FuerJournalisten/01_Presse_und_Hintergrundinformationen/05_Tierarzneimittel/2015/2015_07_28_pi_Antibiotikaabgabemenge2014.html). In Gram-negative bacteria, the enzymatic inactivation by hydrolytic cleavage of the β-lactam ring by β-lactamases is the most important mechanism of resistance to β-lactam antibiotics. Among the various types of β-lactamases, extended-spectrum β-lactamases (ESBLs) are able to confer resistance to penicillins, cephalosporins (including third- and fourth-generation cephalosporins) and monobactams. However, they do not show hydrolytic activity against cephamycins or carbapenems and are inhibited by β-lactamase inhibitors (e.g. clavulanic acid). ESBLs have gained increased attention due to their successful vertical and horizontal dissemination through clones and plasmids harboring ESBL genes, respectively (Cantón et al., 2012). Besides the global dissemination of specific clones or plasmids, there

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are still differences in distributions and prevalences of the ESBL types with regard to different countries, regions, bacterial species, as well as human and animal hosts (EFSA, 2011). Thus, the identification of ESBLs is the first step towards a better understanding of the epidemiology of ESBLs. Therefore, the aim of this study was to identify ESBL-producing *Escherichia coli* isolates collected from diseased food-producing animals in the German national resistance monitoring program GE*RM*-Vet.

2. Materials and methods

2.1. Sampling strategy of the GERM-Vet monitoring program and screening for ESBL-producing isolates

A total of 6849 *E. coli* from diseased food-producing animals, including cattle (n = 2896, 42.3%), pigs (n = 1562, 22.8%) and poultry (n = 2391, 34.9%), were collected in the GE*RM*-Vet monitoring program during the years 2008–2014. This monitoring program is conducted by the Federal Office of Consumer Protection and Food Safety (BVL) in cooperation with more than 30 diagnostic laboratories distributed all over Germany which are involved in sample collection and bacterial isolation. The isolates were selected according to a defined sampling plan. To avoid the inclusion of duplicate isolates, only one isolate per herd/year was included in the program (Wallmann et al., 2003).

Isolates displaying a cefotaxime MIC of ≥ 1 mg/L and/or colonies with typical appearance of ESBL-producing *E. coli* on CHROMagar ESBL plates (Mast Diagnostica, Reinfeld, Germany) were considered as putative ESBL-producers and were subjected to confirmatory tests.

2.2. Confirmatory tests for ESBL-producing isolates, additional antimicrobial susceptibility testing and phylotyping

The confirmation of the ESBL phenotype was performed by disk diffusion using the combination disk test according to the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2016). ESBL-producing isolates were initially investigated by a multiplex PCR assay for the detection of the main β-lactamase/ESBL gene groups bla_{TEM}, bla_{SVH} and bla_{CTX-M} (Gröbner et al., 2009). The identification of the *bla* gene variants was conducted by additional PCR assays which amplified the entire *bla* gene (Hasman et al., 2005; Paauw et al., 2006; Carattoli et al., 2008; Gröbner et al., 2009) and subsequent sequencing of the amplicons. Sequences were analyzed by BLAST (blastn and blastp, http://blast.ncbi.nlm.nih.gov/Blast.cgi) and by comparison with reference sequences obtained from the Lahey Clinic website (http://www.lahey.org/studies/). The bla_{TEM} genes were sequenced in case no other ESBL gene could be found.

Additional antimicrobial susceptibility testing was performed by disk diffusion for the antimicrobial agents: amikacin (AMK, 30 μg), cefepime (FEP, 30 μg), chloramphenicol (CHL, 30 μg) ertapenem (ETP, 10 μg), imipenem (IPM, 10 μg), sulfonamides (SMX, 300 μg), and trimethoprim (TMP, 5 μg) (Oxoid, Basingstoke, UK) (CLSI, 2013, 2015). The *E. coli* ATCC® 25922 was used as a quality control strain. The zone interpretive criteria for the antimicrobial agents were those for Enterobacteriaceae listed in the CLSI document M100-S26 (CLSI, 2016). The minimal inhibitory concentration (MIC) of the isolates was determined by broth microdilution with custom-made microtitre plates (Sensititre®, TREK Diagnostic systems, East Grinstead, UK) according to the

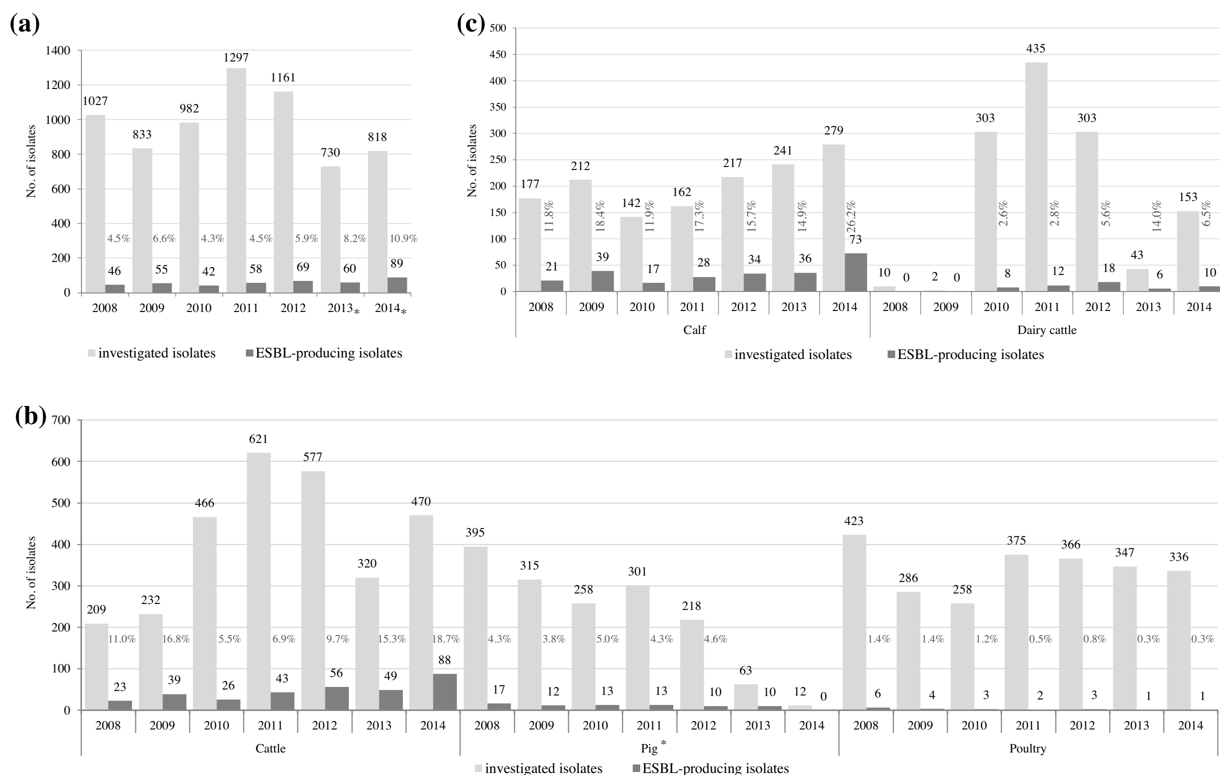

Fig. 1. (a) Overall frequency of ESBL-producing *E. coli* collected from diseased food-producing animals (cattle, pig and poultry) in the GE*RM*-Vet monitoring program over time. (b) Frequency of ESBL-producing isolates according to the specific animal host: cattle, pig or poultry over time. * Due to the rotating sampling plan within GE*RM*-Vet monitoring program, not all pig isolates of 2013 and 2014 were included in the monitoring program and consequently are also not part of this study.

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