Presence of β-lactamases in extended-spectrum-cephalosporin-resistant Salmonella enterica of 30 different serovars in Germany 2005–11

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Objectives: Between 20000 and 35000 cases of salmonellosis are detected annually in Germany, but only a few Salmonella are resistant to third-generation cephalosporins. The German National Reference Centre for Salmonella and other Enterics obtained 150 Salmonella enterica isolates from human infections between 2005 and 2011. In the present study we identified the β-lactamase genes causing resistance to third-generation cephalosporins in these isolates.

Methods: For all isolates serotyping and antimicrobial susceptibility testing were performed. The presence of β-lactamase genes was detected by PCR amplification and sequencing. Isolates with identical serovar and β-lactamase genes were typed by XbaI macrorestriction followed by PFGE. Broth mate conjugation assays and plasmid analysis using S1 nuclease restriction of genomic DNA and subsequent PFGE as well as PCR-based replicon typing were performed for selected isolates.

Results: The 150 isolates were assigned to 30 different serovars, with Salmonella enterica serovar Typhimurium (n = 73; 48.7%) as the most prevalent. Two different AmpC β-lactamase genes (blaCMY-2, n = 8; blaACC-1, n = 6) and various extended-spectrum β-lactamase (ESBL) genes were identified. The majority harboured the blaCTX-M-1 gene (n = 91; 60.7%) followed by blaCTX-M-14 (n = 12; 8.0%) and blaSHV-12 (n = 11; 7.3%). Typing of strains and subsequent comparison with selected Salmonella isolates from livestock revealed the presence of several clones in both humans and livestock.

Conclusions: The wide spread of ESBL and AmpC genes in Salmonella of various serovars is most probably due to transfer of conjugative plasmids. Furthermore, our data indicate the clonal spread of distinct cephalosporin-resistant Salmonella strains from livestock to humans.

Keywords: ESBLs, AmpC, CTX-M-15, CMY-2, antimicrobial resistance

Introduction

Salmonella enterica is a widespread cause of foodborne gastrointestinal infections in Europe, with Salmonella enterica Typhimurium and Salmonella enterica Enteritidis as the most common serovars.1 According to official national statistics, 24454 cases of salmonellosis were reported in Germany in 2011. In recent years the number of reports an emergence of multdrug-resistant Salmonella enterica from humans and animals, especially with resistance to extended-spectrum cephalosporins such as ceftazidime and cefotaxime, has increased worldwide.2–4 Resistance to cephalosporins is mainly due to acquisition of genes encoding extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamases. These β-lactamase genes are mainly plasmid-encoded and transferable into other enterobacterial species. The aim of the present study was the identification of β-lactamases causing resistance to third-generation cephalosporins in Salmonella enterica isolates from humans.

Materials and methods

The National Reference Centre (NRC) for Salmonella and other Enterics in Germany received 23 771 Salmonella enterica isolates, mainly from human infections, between 2005 and 2011. The number of submitted isolates varied in quantity from 2604 isolates in 2011 to 4051 isolates in 2007.
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(average of 3 396 isolates/year). From the NRC database we identified 150 S. enterica isolates with resistance to cefotaxime and/or ceftazidime, and these were included in the present study.

Sero- and phage typing following the guidelines of the International Federation for Enteric Phage Typing (IFEPF) for serovar Salmonella Typhimurium and determination of antimicrobial susceptibilities to 12 different antibiotics (ampicillin, cefotaxime, ceftazidime, cefoxitin, nalidixic acid, ciprofloxacin, gentamicin, amikacin, streptomycin, chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole) by microbroth dilution according to the CLSI criteria were performed. Confirmation of the monophasic variant of Salmonella Typhimurium strains was done by PCR amplification of the pbp2β flagellar gene. All 150 isolates, being resistant to cefotaxime and/or ceftazidime, were tested for ESBL or AmpC production by disc tests (AMPC + ESBL DETECTION SET D68C, MAST Group).

The presence of β-lactamase genes was tested by PCR amplification and sequencing of ESBL genes blag TEM-type, blag SHV-type and blag CTX-M-1/2/9-type and plasmid-mediated ampC genes. The presence of ESBL genes blag TEM-type and blag CTX-M-1-type was tested using primers CTX-M-8-FWD (5' TAAGCCGACCAAGCTTACC-3') and CTX-M-8-REV (5'-GGATCTATTGACGACACGC3'-3') as well as CTX-M-25-FWD (5'-GCCATGTATTGACGACACGC-3') and CTX-M-25-REV (5'-ACCCGTCGCCAGAATCTTG-3'). The ampC genes blag CMY-type and blag ACC-type were sequenced using the primers CMY-FWD (5'-CTGTCGTGCTAGGACCT-3') and CMY-REV (5'- CTGACACGGACAGGTTAG-3'), and ACC-FWD (5'-TACCTGTGCTGCGCAACGCT-3') and ACC-REV (5'-TTTTATACCCGCACTCT-3'), respectively. In isolates with MICs of ciprofloxacin of 0.125 to >64 mg/L the presence of plasmid-mediated quinolone resistance (PMQR) genes (ampC-type genes and aac(6')-Ib-cr) was determined.

Genetic relatedness was determined by XbaI macrorestriction analysis followed by PFGE with interpretation according to the criteria of Tenover et al. Plasmids of selected isolates were transferred by broth mating conjugation into a sodium azide-resistant Escherichia coli K12J53 recipient. Plasmids were characterized using S1 nuclease restriction of genomic DNA and subsequent PFGE as well as PCR-based replicon typing.

### Results and discussion

Within the period of 7 years (2005–11) we found 150 S. enterica isolates that were resistant to third-generation cephalosporins (average of 22 isolates/year). The most frequent of the 30 determined serovars among these 150 isolates were Salmonella Typhimurium (n = 73; 48.7%), including 23 monophasic isolates 4,5,12,12:1- (Table 1), followed by Salmonella Infantis (n = 11; 7.3%), Salmonella Enteritidis (n = 7; 4.7%) and Salmonella Kentucky (n = 6; 4.0%).

The mean rate of resistance to cefotaxime and/or ceftazidime was 0.6%, with a slight but not significant (P = 0.181; Spearman rank correlation) increase from 2005 (0.36%) to 2011 (0.84%). Furthermore, 17 of the 150 isolates showed a combined resistance to ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole (Table S1, available as Supplementary data at JAC Online) and 13 isolates showed an additional resistance to ciprofloxacin.

By PCR and sequencing we confirmed the presence of various ESBL and AmpC β-lactamase genes (Table 2). The most prevalent gene was blag TEM-1 (n = 91; 60.7%), followed by blag SHV-12 (n = 12; 8.0%) and blag TEM-52 (n = 11; 7.3%). In six isolates blag CTX-M-15 was present and single isolates were positive for blag CTX-M-2, blag CTX-M-2 and blag CTX-M-8 (Table 2). The AmpC β-lactamase genes blag CMY-2 and blag ACC-1 were found in eight isolates (seven different serovars) and six Salmonella Bareilly isolates, respectively. Additionally, different PMQR genes [qnrS1, n = 2; qnrA1, n = 2; aac(6')-Ib-cr, n = 1] were found in seven Salmonella Typhimurium and single isolates of serovars Salmonella Agona, Salmonella Kedougou and Salmonella Typhi.

The XbaI macrorestriction analysis revealed identical macrorestriction patterns, indicating a prolonged transmission of an ACC-1-positive Salmonella Bareilly strain in 2005 and the nationwide dissemination of a CTX-M-1-producing Salmonella Typhimurium clone of phage type DT193 (22 isolates) in 2007. Furthermore, we observed the repeated occurrence of ESBL-positive strains of Salmonella Anatum (two isolates), Salmonella Kentucky (four isolates) Salmonella Kapemba (two isolates) and Salmonella Infantis (four isolates).

The 22 isolates of the CTX-M-1-producing Salmonella Typhimurium DT193 clone were received from different hospitals in five German federal states in 2007. We asked the Federal Institute for Risk Assessment in Berlin to send us isolates from livestock that emerged in 2007 and harboured identical ESBLs. By comparative macrorestriction analysis of the Salmonella Typhimurium isolates, we identified this Salmonella Typhimurium DT193 clone again in three faecal samples from pigs isolated in 2006. The Salmonella Typhimurium DT193 clone contained CTX-M-1 ESBL and the TEM-1 β-lactamase. Subsequent plasmid analysis revealed genes blag TEM-1 and blag TEM-1, located on two conjugative plasmids (>35 and 120 kb) belonging to replicon types IncN and IncFII, respectively.

By comparative analyses of further ESBL-producing Salmonella strains from humans and livestock we identified two strains of Salmonella Infantis carrying blag TEM-52 (IncI1 plasmid, 80 kb) and blag TEM-1 (IncI1 plasmid, 100 kb), respectively. Each of these isolates was genetically identical (PFGE and plasmid analysis) to an isolate from a chicken, found in the same period of time. In addition, the only human Salmonella Paratyphi B d-tartrate-positive (dt+) strain of the present study that carried blag TEM-52 (IncI1

### Table 1. Number of ESBL-positive, monophasic Salmonella Typhimurium in human infections in Germany 2005–11

| Phage type | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | Total | b|a|ESBL/AmpC |
|------------|------|------|------|------|------|------|------|------|------------|
| DT193      | —    | 2    | 2    | 3    | 3    | 2    | 3    | 15   | blag CTX-M-1 |
| DT120      | —    | —    | —    | —    | —    | —    | 1    | 1    | blag CTX-M-1 |
| Untypeable | —    | —    | —    | —    | —    | —    | 6    | 6    | blag SHV-12, n = 3; blag TEM-52, n = 1 |
| RDNC       | —    | —    | —    | —    | —    | 1    | —    | 1    | blag CMY-2 |
| Total      | —    | 2    | 2    | 3    | 3    | 4    | 9    | 23   | —            |

RDNC, reacts but does not conform with any phage type.
plasmid, 80 kb) showed an XbaI macrorestriction pattern identical to those of four TEM-S2-producing Salmonella Paratyphi B (dT+) isolates from poultry.

Our study data demonstrate that the majority (48.7%) of ESBL- and AmpC-producing human S. enterica isolates were Salmonella Typhimurium, which represents one of the most common serotypes in Europe. Among these Salmonella Typhimurium we identified 23 (31.5%) isolates as the monophasic variant 4,[5],12:i:-. The high percentage of ESBL-positive monophasic strains could result from the generally increasing number of monophasic Salmonella Typhimurium according to the data from the NRC database in Germany (n=82 in 2005 to n=621 in 2011) and all over Europe. Furthermore, the present study showed that the majority of strains harboured bla<sub>CTX-M</sub>-like enzymes (n=116; 77.3%) with CTX-M-1 as the most prevalent ESBL type in human S. enterica isolates; similar results were found in Salmonella and E. coli from animals, especially poultry. Moreover, detailed molecular analyses revealed the emergence of partly the same plasmid-encoded ESBL and AmpC types (e.g. CTX-M-1, TEM-52 and CMY-2) as described in a previous study of S. enterica isolates from German livestock animals. In contrast, ESBL type CTX-M-15, which is highly prevalent in human nosocomial E. coli, was identified only in six human S. enterica isolates, including a Salmonella Typhi isolate from a patient from abroad. The comparison of the present results with international data confirmed that CTX-M-1 ESBL and CMY-2 AmpC are prevalent enzymes in third-generation-cephalosporin-resistant Salmonella worldwide. In Spain bla<sub>CTX-M-1</sub> was recently described on IncI1/N plasmids (100 kb), indicating a possible transnational spread of resistant Salmonella clones or plasmids.

A possible transmission route between livestock and humans via the food chain could be postulated in this study for a CTX-M-1-positive Salmonella Typhimurium DT193 strain from pigs as well as Salmonella Infantis and Salmonella Paratyphi B (dT+) strains with CTX-M-1 and/or TEM-52 from poultry. Plasmid-mediated resistance gene transfer among S. enterica and other Enterobacteriaceae might contribute to further spread of ESBL and AmpC β-lactamase genes. Therefore, there is an urgent need for further detailed comparisons of third-generation-cephalosporin-resistant S. enterica from humans, animals and foods to find and evaluate all routes of dissemination.

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### Transparency declarations
None to declare.

### Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxford-journals.org/).

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