

High prevalence of extended-spectrum- β -lactamase-producing Enterobacteriaceae in organic and conventional retail chicken meat, Germany

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Objectives: To determine the prevalence of extended-spectrum β -lactamase (ESBL) production in Enterobacteriaceae in retail chicken meat in Germany.

Methods: A total of 399 chicken meat samples from nine supermarket chains, four organic food stores and one butcher's shop in two geographically distinct regions (Berlin and Greifswald) were screened for ESBL production using selective agar. Phenotypic ESBL isolates were tested for *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{SHV} genes using PCR and DNA sequencing. Antibiotic coresistances were determined and strain typing was performed using PCR-based phylogenetic grouping and XbaI-PFGE.

Results: A total of 185 confirmed ESBL isolates were obtained from 175 samples (43.9%) from all tested sources. The majority of isolates were *Escherichia coli* producing ESBL types SHV-12 (*n*=82), CTX-M-1 (*n*=77) and TEM-52 (*n*=16). No differences could be observed in the prevalence of ESBL producers between organic and conventional samples. 73.0% of the ESBL producers showed coresistance to tetracycline, 35.7% to co-trimoxazole and 7.6% to ciprofloxacin. Strain typing of selected *E. coli* isolates from Berlin revealed identical macrorestriction patterns for several isolates from samples taken from the same stores.

Conclusions: This is the first comprehensive study from Germany showing a high prevalence of TEM-, CTX-M- and SHV-type ESBLs in Enterobacteriaceae isolated from retail chicken meat. The high rate of coresistance to different classes of antibiotics in the ESBL producers might reflect the common veterinary usage of these and related substances. There is an urgent need to further evaluate the role of poultry in the transmission of highly resistant ESBL-producing bacteria in humans.

Keywords: ESBLs, poultry, coresistance

Introduction

Extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae can cause life-threatening infections in hospitalized patients, but can also be isolated from outpatients or healthy persons.^{1,2} The origin of ESBL producers in the different health-care or community settings is of particular interest for the implementation of effective infection control measures. It was shown that ESBL producers can be isolated from chicken faeces or meat in Portugal, Belgium, Spain and Great Britain.^{1,3} Recently, Overdevest *et al.*³ and Leverstein-van Hall *et al.*⁴ demonstrated a similar distribution of ESBL drug resistance genes among isolates from

retail chicken meat and poultry, and isolates from colonized as well as infected humans in the Netherlands. Thus, the food chain might represent a substantial source for the transmission of ESBL genes or ESBL-producing strains to the human intestinal bacterial flora.

There has been no comprehensive study on the distribution of ESBL producers in food in Germany. We therefore investigated the prevalence of ESBL-producing Enterobacteriaceae in fresh retail chicken meat from different supermarket chains and organic food stores in two different geographical regions of Germany.

Methods

Sampling strategy

Between 16 and 26 August 2011, 199 chicken breasts and legs were collected from six supermarket stores, two organic food stores and one butcher's shop in Berlin. Furthermore, between 24 October and 15 November 2011, 200 chicken breasts and legs were collected from seven supermarket stores and two organic food stores in Greifswald (northern Germany). The 13 supermarket stores in Berlin and Greifswald represent nine different supermarket chains.

Isolation of bacteria and phenotypic characterization

Meat samples (5 g) from each package were homogenized and incubated overnight at 37°C in 25 mL of tryptic soy broth. The broth (100 µL) was placed on a chromogenic agar plate selective for ESBLs (CHROMagar ESBL, Mast Group, Reinfeld, Germany). After overnight incubation, colonies with distinct morphological appearance were further investigated. Species identification and detection of antimicrobial susceptibilities were performed using standard biochemical tests (API 20 E[®], bioMérieux, Nürtingen, Germany) and the Vitek 2 system (Vitek 2 GN-ID card and Vitek 2 card 111, bioMérieux). For phenotypic confirmation of ESBL production, the MASTDISCS[™] ID ESBL Set CPD10 (Mast Group) was used.

Genotypic characterization

All isolates from a single sample showing an ESBL phenotype were screened for different ESBL genes (*bla*_{TEM-type}, *bla*_{SHV-type} and *bla*_{CTX-M-type}) by multiplex PCR. Sequencing of the complete genes in β-lactamase-positive isolates was performed as described previously.^{5,6} Furthermore, we determined the phylogenetic group of selected *Escherichia coli* isolates of several samples from Berlin using PCR and XbaI-macrorestriction analysis followed by PFGE, according to the Tenover criteria.^{7,8}

Statistical analyses

A multivariable logistic regression analysis (MLRA) was performed for testing the significance of ESBL prevalence. Adjusted ORs and corresponding 95% CIs were calculated based on general estimating equation (GEE) models. The type of meat (leg or breast), store (supermarket/organic store/butcher) and chain (14 different types: 9 supermarket chains, 4 organic food stores and 1 butcher's shop) were investigated in an MLRA. Since isolates from one store are not statistically independent, adjusted ORs were estimated based on GEE models, which account for this clustering effect.⁹ In the multivariable analysis, a stepwise forward variable selection was used with $P < 0.05$ (χ^2 test, type III) for including a variable in the model and $P \geq 0.05$ for excluding a variable. Multivariable analyses were performed using IBM SPSS statistics 19.0.

Results

Prevalence of ESBL-producing bacterial species

Out of 199 samples from nine stores in Berlin, we identified 81 putative ESBL-producing Enterobacteriaceae isolates from 80 samples [*E. coli* 93.83% ($n=76$), *Serratia fonticola* 3.70% ($n=3$), *Escherichia fergusonii* 1.23% ($n=1$) and *Enterobacter cloacae* 1.23% ($n=1$)]. PCR and sequencing of β-lactamase genes revealed 75 ESBL-producing isolates (73 *E. coli*, 1 *E. fergusonii* and 1 *E. cloacae*). One sample contained two ESBL-producing *E. coli* strains.

Out of 200 analysed chicken meat samples from nine stores in Greifswald, we isolated 155 putative ESBL-producing bacteria

from 123 samples [*E. coli* 87.10% ($n=135$), *S. fonticola* 12.26% ($n=19$) and *Proteus mirabilis* 0.65% ($n=1$)]. The sequence analysis of β-lactamase genes in these 155 isolates confirmed 110 ESBL producers (108 *E. coli*, 1 *S. fonticola* and 1 *P. mirabilis*). Eight samples contained more than one ESBL-producing strain.

In total, 37.2% ($n=74$; range 12%–70%) of samples purchased in Berlin and 50.5% ($n=101$; range 15%–68%) of samples from Greifswald were contaminated with ESBL-producing bacteria. Type of meat (leg or breast) and type of supermarket chain (14 different types) were independently associated with ESBL-producing bacteria. Interestingly, we found a 57% lower risk for ESBL prevalence in legs adjusted by type of chain and cluster effects by store [adjusted OR for leg was 0.43 ($P=0.03$; CI 95%: 0.20–0.94)]. Additionally, there were significant differences between the different supermarket chains (data not shown). More importantly, no significant difference in the prevalence of ESBL-producing bacteria was observed between samples isolated from supermarkets, organic food stores and the butcher's shop, neither in the univariable GEE model nor in the multivariable GEE model together with type of meat (leg or breast).

ESBL gene distribution, phylogenetic grouping and coresistance

Molecular analysis of β-lactamase genes revealed no significant differences between isolates from Berlin and Greifswald (Figure 1). The most prevalent ESBL genes (total $n=187$) were *bla*_{SHV-12} (43.9%, $n=82$), followed by *bla*_{CTX-M-1} (41.2%, $n=77$) and *bla*_{TEM-52} (8.6%, $n=16$). In samples from Berlin, we additionally detected *bla*_{CTX-M-2} (3.2%, $n=6$) and *bla*_{SHV-2} (0.5%, $n=1$). In samples from Greifswald, the ESBL genes *bla*_{SHV-2A} (2.1%, $n=4$) and *bla*_{CTX-M-65} (0.5% $n=1$) could be identified (Figure 1). In two strains we identified the ESBL gene combinations *bla*_{CTX-M-1}/*bla*_{TEM-52} (*E. coli*) and *bla*_{CTX-M-1}/*bla*_{SHV-12} (*E. fergusonii*). It is interesting that we found a similar ESBL gene distribution in isolates from conventional and organic meat samples. In the latter, ESBL types CTX-M-1, CTX-M-2 and SHV-12 were the most dominant ones (data not shown).

Typing of selected *E. coli* isolates ($n=30$) from samples from six supermarket chains, two organic food stores and the butcher's shop in Berlin was performed by PCR-based phylogenetic grouping and XbaI-PFGE. The analyses revealed that the *E. coli* isolates from several samples taken from the same supermarket, organic food store or butcher's shop showed identical macrorestriction patterns (Figure 2). Furthermore, one distinct CTX-M-1-producing *E. coli* (phylogenetic group A) strain was confirmed in different samples from three supermarket chains, indicating the clonal transfer of this strain.

We found that 73.0% ($n=135$) of the ESBL-positive isolates from Berlin and Greifswald were additionally co-resistant to tetracycline, 35.7% ($n=66$) were resistant to co-trimoxazole, 7.6% ($n=14$) to ciprofloxacin and 2.7% ($n=5$) to aminoglycosides. However, 45.3% ($n=34$) of the isolates from Berlin showed increased MIC values of ciprofloxacin (0.25–1 mg/L). A screening for plasmid-mediated quinolone resistance genes was negative, indicating mutations of chromosome-encoded genes (*parC* and *gyrA*) might cause this reduced susceptibility to ciprofloxacin.

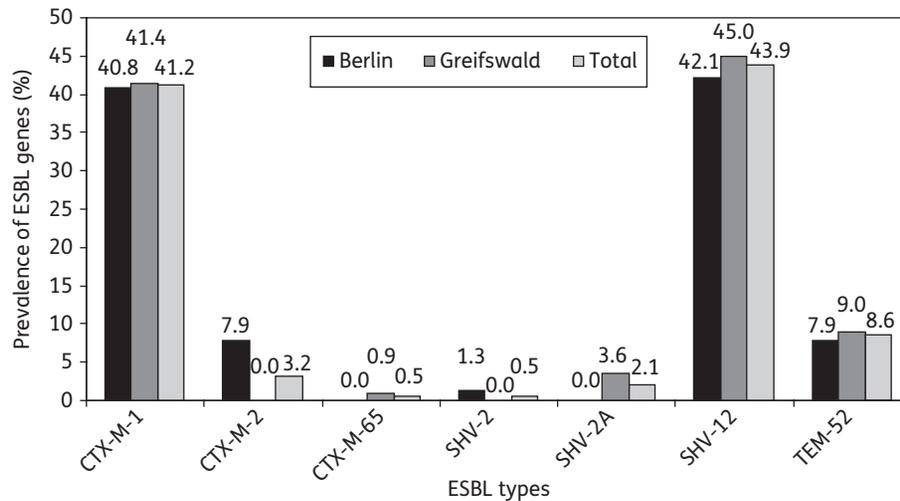


Figure 1. Distribution of single ESBL types in Enterobacteriaceae isolates from retail chicken meat samples purchased in Greifswald and Berlin. The prevalence of the ESBL genes (%) was calculated for Greifswald (n=111), Berlin (n=76) and for the total of both groups (n=187).

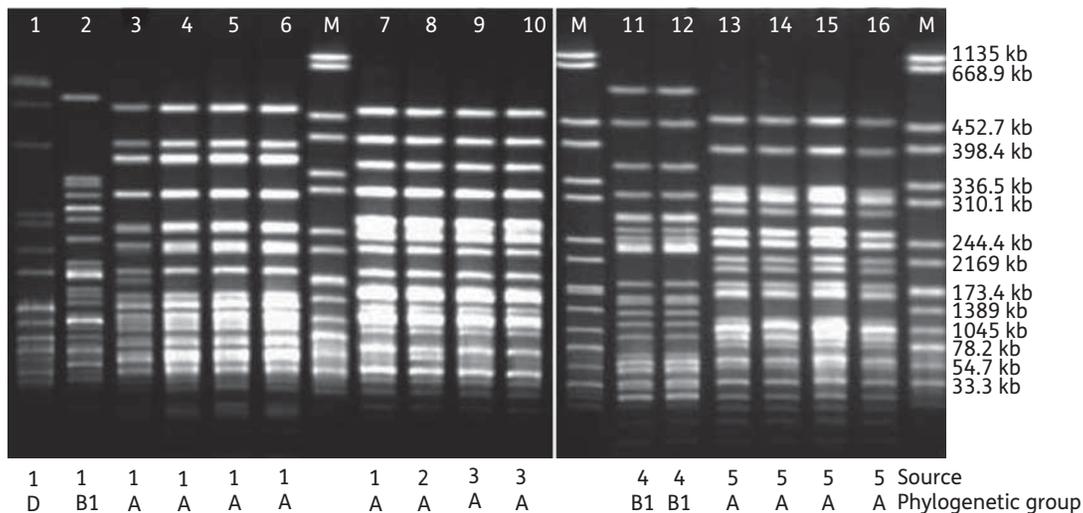


Figure 2. XbaI macrorestriction patterns (PFGE) of selected isolates from different samples and sources from Berlin. Lane M, *Salmonella enterica* Braenderup standard; lanes 1 and 2, *E. coli* isolates from different samples from Supermarket 1 with CTX-M-1; lanes 3–6, *E. coli* isolates from different samples from Supermarket 1 with CTX-M-1+TEM-135; lanes 7–10, *E. coli* isolates from samples from Supermarkets 1, 2 and 3 with CTX-M-1; lanes 11 and 12, *E. coli* isolates from different samples from an organic food store (4) with CTX-M-2; lanes 13–16, *E. coli* isolates from different samples from the butcher’s shop (5) with CTX-M-1.

Discussion

The present study shows an alarmingly high prevalence (43.9%) of ESBL-producing Enterobacteriaceae in retail chicken meat from different regions in Germany. Most of our ESBL isolates harbour the ESBL genes *bla*_{SHV-12}, *bla*_{CTX-M-1} and *bla*_{TEM-52}, which have also been commonly found in poultry isolates from the Netherlands, Belgium, France and England, but also in human isolates from Germany and the Netherlands.^{1,3,10} However, the rate of ESBL-producing bacteria in our study was half the rate found in recent studies in the Netherlands.^{3,11} One might speculate that the increased prevalence of ESBL producers in the Netherlands is directly associated with the very

high usage of antibacterial agents in animal farms in this country, which is more than twice as high as described for Germany and most other European countries.^{3,12} The prevalent coresistance of the ESBL-producing *E. coli* from our study to tetracycline and co-trimoxazole has also been reported for ESBL-producing isolates from patients and healthy persons.^{13,14} Interestingly, these classes of antibiotics seem to be mainly used in animal farms, in addition to β-lactam antibiotics, quinolones, aminoglycosides or macrolides.¹² Noteworthy, we found also a high prevalence (36%) of ESBL-producing bacteria in organic chicken meat, which confirms a recent report from the Netherlands.¹¹ Regarding the typing results of the present study, we

speculate that the ESBL contamination might occur either through ESBL-colonized 1-day-old chicks that are introduced into organic farms, or through cross-contamination between conventional and organic flocks during the rearing process or slaughtering or finally through an ESBL-contaminated environment.¹¹

Recently, Leverstein-van Hall et al.⁴ showed that 19% of human ESBL-positive *E. coli* isolates in the Netherlands contained ESBL genes on plasmids that were genetically indistinguishable from those of poultry isolates. In addition, Vincent et al.¹⁵ described *E. coli* food isolates that were indistinguishable from *E. coli* isolates causing urinary tract infection. Further detailed investigations of ESBL strains and the *bla*_{ESBL}-carrying plasmids in human and animal isolates are needed to determine the role of the food chain in the transmission of ESBL-producing bacteria in Germany.

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Transparency declarations

None to declare.

Author contributions

A. K., P. G., W. W. and I. S. planned the study, A. B., A. K., K. K., K. S., V. B. and K. B. conducted the microbiological analysis of the retail chicken samples, C. K. and Y. P. performed the molecular typing of strains and ESBL genes, C. K., Y. P. and F. S. analysed as well as interpreted the data, and C. K., Y. P., A. K. and I. S. prepared the manuscript.

References

- 1 Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008; **13**: pii=19044.
- 2 Oteo J, Navarro C, Cercenado E et al. Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. *J Clin Microbiol* 2006; **44**: 2359–66.
- 3 Overdeest I, Willemsen I, Rijnsburger M et al. Extended-spectrum β -lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. *Emerg Infect Dis* 2011; **17**: 1216–22.
- 4 Leverstein-van Hall MA, Dierix CM, Cohen Stuart J et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect* 2011; **17**: 873–80.
- 5 Grobner S, Linke D, Schutz W et al. Emergence of carbapenem-non-susceptible extended-spectrum β -lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. *J Med Microbiol* 2009; **58**: 912–22.
- 6 Pfeifer Y, Matten J, Rabsch W. *Salmonella enterica* serovar Typhi with CTX-M β -lactamase, Germany. *Emerg Infect Dis* 2009; **15**: 1533–5.
- 7 Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; **66**: 4555–8.
- 8 Tenover FC, Arbeit RD, Goering RV et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.
- 9 Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics* 1988; **44**: 1049–60.
- 10 Canton R, Novais A, Valverde A et al. Prevalence and spread of extended-spectrum β -lactamase-producing Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2008; **14** Suppl 1: 144–53.
- 11 Cohen Stuart J, van den Munckhof T, Voets G et al. Comparison of ESBL contamination in organic and conventional retail chicken meat. *Int J Food Microbiol* 2012; **154**: 212–4.
- 12 Grave K, Torren-Edo J, Mackay D. Comparison of the sales of veterinary antibacterial agents between 10 European countries. *J Antimicrob Chemother* 2010; **65**: 2037–40.
- 13 Schwaber MJ, Navon-Venezia S, Schwartz D et al. High levels of antimicrobial coresistance among extended-spectrum- β -lactamase-producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2005; **49**: 2137–9.
- 14 Geser N, Stephan R, Korczak BM et al. Molecular identification of extended-spectrum- β -lactamase genes from Enterobacteriaceae isolated from healthy human carriers in Switzerland. *Antimicrob Agents Chemother* 2011; **56**: 1609–12.
- 15 Vincent C, Boerlin P, Daignault D et al. Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg Infect Dis* 2010; **16**: 88–95.