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 blaTEM, blaCTX-M and blaSHV genes using PCR and DNA sequencing. Antibiotic co-resistances 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 co-resistance 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 co-resistance 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, co-resistance

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 healthcare 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, Overdvest 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 resistances 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. 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.

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. In the multivariable analysis, a stepwise forward variable selection was used with P < 0.05 (χ² test, type III) for including a variable in the model and P ≥ 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 co-resistance

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 blaSHV-12 (43.9%, n = 82), followed by blaCTX-M-1 (41.2%, n = 77) and blaTEM-52 (8.6%, n = 16). In samples from Berlin, we additionally detected blaCTX-M-2 (3.2%, n = 6) and blaSHV-2 (0.5%, n = 1). In samples from Greifswald, the ESBL genes blaSHV-2A (2.1%, n = 4) and blaCTX-M-65 (0.5% n = 1) could be identified (Figure 1). In two strains we identified the ESBL gene combinations blaCTX-M-1/blaTEM-32 (E. coli) and blaCTX-M-5/blaSHV-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 coresistant 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.
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 \(\text{bla}^{\text{SHV-12}}, \text{bla}^{\text{CTX-M-1}}\) and \(\text{bla}^{\text{TEM-52}},\) 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.\(^{3,10}\) 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 co-resistance 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.\(^{3,14}\) Interestingly, these classes of antibiotics seem to be mainly used in animal farms, in addition to \(\beta\)-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

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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 different 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.
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.\textsuperscript{11}

Recently, Leverstein-van Hall et al.\textsuperscript{4} showed that 19% of human ESBL-positive \textit{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.\textsuperscript{15} described \textit{E. coli} food isolates that were indistinguishable from \textit{E. coli} isolates causing urinary tract infection. Further detailed investigations of ESBL strains and the \textit{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