**Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry**

Beatriz Guerra¹, Ernst Junker¹, Andreas Schroeter¹, Burkhard Malorny¹, Simone Lehmann² and Reiner Helmuth¹*

¹National Salmonella Reference Laboratory (Berlin) and ²National Escherichia coli Reference Laboratory (Dessau), Federal Institute for Risk Assessment (BfR), P.O. Box 480447, D-12254 Berlin, Germany

Received 1 April 2003; returned 23 April 2003; revised 2 June 2003; accepted 9 June 2003

**Objective:** Phenotypic and genotypic characterization of the antimicrobial resistance of German *Escherichia coli* strains isolated during 1999–2001 from cattle, swine and poultry.

**Materials and methods:** Three hundred and seventeen isolates were tested for their resistance to 17 antimicrobial agents by broth microdilution. Resistant strains were screened by molecular methods for resistance genes, integrons and mutations in quinolone-resistance determining regions.

**Results:** Resistance was found in 40% and multiresistance in 32% of the strains. The resistance was significantly higher in isolates from poultry (61%) and swine (60%) than from cattle (25%) (P < 0.01). The most prevalent resistances were to sulfamethoxazole, tetracycline, streptomycin, ampicillin and spectinomycin (30–15%). For each antibiotic, the predominant resistance genes were: ampicillin, *bla*TEM-like (92%); chloramphenicol, *catA* (68%) and *cmlA1*-like (36%); gentamicin, *aac(3)-IV* (60%); kanamycin, *aphA1* (100%); streptomycin, *aadA1*-like (61%) and *strA/B* (59%); sulfamethoxazole, *sul2* (66%), *sul1* (42%) and *sul3* (14%); tetracycline, *tet(A)* (66%) and *tet(B)* (42%); and trimethoprim, *dfrA1*-like (77%), *dfrA17* (13%) and *dfrA12* (7%). Class 1 integrons were found in 30% of the strains. They carried *dfrA1-aadA1a* (40%), *aadA1a* (29%), *sat1-aadA1a* (16%), *dfrA17-aadA5* (11%), *oxa1-aadA1a* (5%) and *dfrA12-aadA2* (3%). Eleven percent of the strains were resistant to nalidixic acid. Of these, 61% presented a reduced susceptibility to ciprofloxacin (MIC = 0.12–2 mg/L) and single mutations in *gyrA* or *gyrA* and *parC* genes, and 39%, full resistance to ciprofloxacin (MIC ≥ 4 mg/L) and double and single mutations in *gyrA* and *parC*, respectively.

**Conclusion:** The study gives baseline information on the magnitude of the resistance problem and its genetic background in contemporary German *E. coli* from food-producing animals.

Keywords: resistance genes, quinolones, integrons

---

**Introduction**

Isolates of *Escherichia coli* can be non-pathogenic commensals or human and/or animal pathogens. They are classified as enterotoxigenic, enteropathogenic, enteroinvasive, or enterohaemorrhagic according to the presence of specific virulence factors.¹ Some isolates (i.e. *E. coli* shiga-like toxin producers, STEC) are zoonotic agents which can cause serious intra- and extra-intestinal diseases like diarrhoea, haemorrhagic colitis (HC) and haemolytic-uraemic syndrome (HUS). Although antimicrobial therapy is generally not required, the emergence of strains showing multiresistance to several antimicrobial drugs is a public health concern.² As a matter of fact, *E. coli* from livestock is exposed to a great selective pressure because in some countries, more than half of the antimicrobial agents are used in food-producing animals.³,⁴ Consequently, resistance is increasing and various resistance determinants have been described. Resistance genes can spread on mobile genetic elements like plasmids, transposons and integrons.⁵

Integrons are gene expression systems that incorporate gene cassettes and make them functional. Class 1 integrons are the most frequently detected ones among Enterobacteriaceae. They comprise two conserved segments (CS) flanking a variable region (VR) where the gene cassettes can be located. The 5’CS consists of an integrase gene (*intI*) and specific insertion site (*attI*). The 3’CS usually contains a *qacEΔ1* and a *sul1* gene (encoding quaternary ammonium compound and sulphonamide resistance).⁵

---

*Corresponding author. Tel: +49-30-8412-2233; Fax: +49-30-8412-2953; E-mail: r.helmuth@bfr.bund.de

Published by Oxford University Press.
To generate baseline data to be used in future risk assessment of antimicrobial resistance, a number of surveillance systems on the local, continental or global scale have been initiated. Among the species proposed for surveillance is E. coli. Consequently, the prevalence and molecular basis of antimicrobial resistance in E. coli strains from humans, livestock and food have been investigated in several countries. This paper describes the antimicrobial drug resistance situation of German E. coli isolates obtained from cattle, poultry and swine (livestock and food) during 1999–2001.

Materials and methods

The study comprised 317 epidemiologically unrelated German E. coli strains isolated during 1999–2001 from cattle, swine and poultry. No distinction was made between isolates from living animals and their corresponding food products. One hundred and eighty strains originated from cattle, 95 from poultry and 42 from swine. These were selected from approximately 800 isolates submitted to the German E. coli National Reference Laboratory (Dessau-Berlin) annually. To avoid replicate isolates only one isolate from the same geographic location/laboratory showing a particular serotype and biotype was selected. The selected strains were heterogeneous in respect to: geographic locations (14 of the 16 German Federal Länder, serotypes (70 O-groups, only five represented by more than 10 strains), biochemical properties (119 patterns) and sources (livestock 199, food 118).

The MICs of 17 antimicrobial agents were assessed by the NCCLS broth microdilution method. The NCCLS recommended breakpoints were used, except for ceftiofur (≥8 mg/L), colistin (≥216 mg/L), florfenicol (≥23 mg/L), neomycin (≥16 mg/L), spectinomycin (≥128 mg/L) and streptomycin (≥32 mg/L) (DANMAP7 recommended breakpoints). In the phenotypic analysis, isolates with intermediate MICs were not considered as resistant.

The detection of antimicrobial resistance genes, class 1 integrons and mutations in the quinolone-resistance determining region (QRDR) was carried out by PCR and DNA sequencing. The identification of resistance genes followed a two-step screening approach. All isolates showing full or intermediate resistance to a certain antimicrobial were tested for the corresponding resistance genes shown in Table 1. Those strains that were PCR negative were screened for additional resistance determinants (end of Table 1). Information about the primers used can be requested from the authors. Plasmid DNA was extracted by an alkaline denaturation method. The location of class 1 integrons was determined by Southern-hybridization with intI1 and sul1 probes.

Results and discussion

Forty percent of all strains were resistant to at least one of the antimicrobial agents tested, 8% were monoresistant and 32% multi-resistant (2–8 resistance determinants). The resistance prevalence in cattle (25%) was significantly lower than in swine and poultry (60–61%) (P < 0.01, χ²). The prevalences of individual resistances were: sulfamethoxazole, tetracycline and streptomycin (30–28%), ampicillin and spectinomycin (19–15%), nalidixic acid, kanamycin, trimethoprim, neomycin, trimethoprim–sulfamethoxazole and chloramphenicol (11–8%), ciprofloxacin and co-amoxiclav (4–3%), gentamicin, colistin and florfenicol (≤2%) and ceftiofur (0%). Intermediate resistance to streptomycin, florfenicol, co-amoxiclav or chloramphenicol was also detected (1% each). The 128 drug-resistant strains were grouped into 75 different resistance-phenotypes (only 10 represented by more than two strains). The most frequent were streptomycin-sulfamethoxazole-tetracycline (8% of the resistant strains), tetracycline (7%), and streptomycin–spectinomycin–sulfamethoxazole-tetracycline (5%). Among the strains isolated from cattle, only resistance to streptomycin, sulfamethoxazole or tetracycline reached frequencies ≥14%. Strains isolated from swine and poultry, in contrast, showed resistance to these antimicrobial agents and ampicillin (≥30%) and to kanamycin-neomycin (17%). In regard to other resistances the prevalence of spectinomycin and chloramphenicol (31% and 19%) resistance in swine, and nalidixic acid (33%), ciprofloxacin (14%), trimethoprim (22%) and trimethoprim–sulfamethoxazole (21%) resistance in poultry, were noteworthy. These differences could be related to the different antibiotic regimes used for the different antimicrobial agents and livestock species. Within each animal species, there was no significant difference in the phenotypes of livestock or food isolates (data not shown).

The molecular investigations on the underlying resistance mechanisms showed that identical resistance phenotypes were based on different genes (Table 1): ampicillin (blaTEM-1-like and blaOXA-1-like genes), chloramphenicol (cmiA-like and cata genes), streptomycin-spectinomycin (aadA1a, aadA2, aadA5 and strA/B genes), sulfamethoxazole (sul1, sul2 and sul3 genes), tetracycline (tetA, tetB and tetD genes), and trimethoprim (dfrA1-like, dfrA7, dfrA12, dfrA14 and dfrA17 genes). Some of the genes (blaTEM-1-like, cata, aac(3)-IV, aphA1, aadA1-like, strA/B, sul2, tetA (and dfrA1-like) were widely spread (frequency >60%) among the resistant strains. In seven cases (three for ampicillin, two for chloramphenicol and two for gentamicin), the genes responsible for resistance could not be identified, indicating other possible resistance mechanisms. In 49 cases, more than one gene encoding the same resistance was present in one strain (the tandem strA/B and aadA1-like in 17 strains, sul1 and sul2 in 13, tetA (and tetB in eight, sul3 and sul2 or sul1 in four and three, cata and cmlA in three and dfrA1-like and dfrA17 in one). The newly described sul3 gene (accession number AJ459418) was found in Swiss E. coli isolates from pigs was detected in 14% of the sulfamethoxazole-resistant isolates (eight strains from swine, four from poultry and one from cattle). No floR, blaCAMB2 (pse-1) or tet(G) genes, generally associated with a resistance island in Salmonella Typhimurium DT104-clone, were found, although floR has been found in plasmids of E. coli. About 30% (38 strains) of all resistant isolates investigated carried class 1 integrons. They were more prevalent in strains isolated from poultry (36% of resistant strains from poultry) and swine (32%) than from cattle (20%). These strains showed different resistance phenotypes, but all of them were resistant to streptomycin. Six types of integrons (five, four and three in swine, cattle and poultry, respectively) were detected. Gene cassettes which conferred resistance to streptomycin/spectinomycin (aadA1a, aadA2 and aadA7) were present in all integron-positive strains, and to trimethoprim (dfrA1, dfrA17 and dfrA12), in 53% of the cases. Fifteen strains (40% of integron positive) carried integrons (VR-amplicons of about 1600 bp) with dfrA1-aadA1a, and showed trimethoprim-resistance as well. However, two of the strains were sulfamethoxazole-susceptible, and their integrons lacked sul1 and qacEAl/sul1. Additional resistances were also found. Eleven and six strains (29% and 16%) carried integrons (amplicons of 1000 and 1900 bp) with aadA1a and sat1 (streptothricin-resistance)-aadA1a, respectively. All these strains had the phenotype streptomycin-spectinomycin-sulfamethoxazole in common, but additional resistances were also found. Four strains (11%) carried integrons (amplicons of 1700 bp) with dfrA17-aadA5, and were of the trimethoprim-trimethoprim–sulfamethoxazole-streptomycin-sulfamethoxazole-ampicillin-chloramphenicol-tetracycline phenotype. Again additional resistances were found as well. Two strains (5%) carried integrons (amplicons of 2000 bp) with
the $\text{bla}_\text{OXA-1}$-aadA1a, and showed the phenotype ampicillin-
carboxaclav-streptomycin-spectinomycin-sulfamethoxazole-kamycin-
nonemycin in combination with additional resistances. One of these
also carried the integron with $\text{dfrA1}$-aadA1a. The last strain (3%)
carried an integron (amplon of 1850 bp) with $\text{dfrA12}$-aadA2
and showed the trimethoprim-trimethoprim–sulfamethoxazole-strepto-
mycin-spectinomycin-sulfamethoxazole-ampicillin-tetracycline phenot-
type. The integron lacked the $\text{qacE}1$ and sul1 genes. However, the
strain carried a sul3 gene which probably encoded the sulfamethoxa-
zoze resistance. In 32 strains, the integron probes hybridized to plasmids of >50 kb. In two strains, more than one plasmid carried an
integron.

The results presented show the wide distribution of integrons on
resistance plasmids of $E. coli$ and emphasize their high potential to
contribute to the efficient spread of antibiotic resistance.\footnote{The finding
of strains which lacked $\text{qacE}1$/sul1 (two) or only sul1 (one), was
interesting, and in fact, defective integrons have been described\footnote{including strains showing intermediate resistance.}
lacking some of these genes usually located in the 3'CS.

Table 2 shows the amino acid changes in the QRDRs of the 36
nalidixic acid-resistant strains. Fourteen of them (13 from poultry)
exhibited resistance to ciprofloxacin (MIC $\geq$ 4 mg/L), and showed
the $\text{gyrA}$ and the third affecting
$\text{parC}$. The other 22 strains (18 from poultry) exhibited reduced
susceptibility to ciprofloxacin (MIC 0.12–2 mg/L). They showed
mutations in the Ser-83 of the $\text{gyrA}$ gene, or a double mutation affecting $\text{gyrA}$ and $\text{parC}$ (only one isolate).
The fact that most of the nalidixic acid-resistant strains (31 of
36) are of poultry origin is remarkable. It points to the selective poten-
tial of fluoroquinolones, in commercial poultry (chicken and turkey)
production.\footnote{The integron}\footnote{including strains showing intermediate resistance.}
In conclusion, the data presented support the exposure assessment within the scientific risk analysis of antimicrobial drug resistance and highlight the need for the prudent use of antimicrobials in animal husbandry.

Acknowledgements

We thank P. Gallien, M. Timm and H. Steinrück (NRL-E. coli Dessau and Berlin, BfR) for the E. coli strains, and for their expert comments in the E. coli field. We also thank D. Mazel (Institut Pasteur, France) and V. Perreten (University of Bern, Switzerland) for the pSK1(intI1, sul1) and pUVP4401 (sul3) plasmids, respectively, B. Hoog (NRL-Salm, Berlin) and G. Hultsch (NRL-E. coli, Berlin) for their very helpful technical assistance, and L. Beutin, M. C. Mendoza and A. Miko for the critical review of the manuscript. This work was supported by grants from the German Ministry of Consumer Protection and Agriculture (BMVEL, AZ:1000-WK-17/00) and the BfR, formerly BgVV (Ref. Fg.501–28/1322–136).

References