Full Length Research Paper

Resistance patterns and detection of resistance genes in *Escherichia coli* isolated from diarrheic calves in Northeastern China

Yue Jiang¹,² and Xiu-Ying Zhang¹,³*

¹Harbin Veterinary Research Institute, Heilongjiang 150001. People's Republic of China.
²Liaoning Medical University, Liaoning 121000. People's Republic of China.
³Northeast Agricultural University, Heilongjiang 150030. People's Republic of China.

Accepted 19 December, 2012

The aim of this study was to investigate the antimicrobial resistance and to characterize the implicated genes in *Escherichia coli* isolated from diarrheic calves during 2008-2011. A total of 104 samples were tested to 15 antimicrobial agents with disk-diffusion method. High resistance to amoxicillin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole and doxycycline was detected. A total of 13 out of the 16 resistance genes were searched in this study. The principal mechanisms of resistance found in the 104 calf isolates were *bla* TEM (84.6%), *aadA1* (73.3%), *cmlA* (46.2%), *tet(B)* (79.8%), *sulI* (74.0%) and *dhfrV* (54.8%). One kind of amino acid change in *gyrA* (four Ser→Leu) and sixteen in *parC* (twelve 80Ser→Arg, four 80Ser→Ile) were identified in the 20 fluoroquinolone-resistant isolates. According to pulsed field gel electrophoresis (PFGE) results, clonal dissemination of multi-drug resistance (MDR) strains played an important role in the severe resistant situation among diarrheic neonatal calves in northeastern China.

Key words: *Escherichia coli*, calf, antimicrobial resistance, resistance genes, clonal dissemination.

INTRODUCTION

*Escherichia coli* is a common inhabitant of intestinal tract of humans and animals, and can be implicated in many human and animal infectious diseases. Certain pathogenic *E. coli* strains are associated with neonatal or post-weaning domestic animal diseases such as diarrhoea of piglets, calves, lambs and edema of piglets (Vihan et al., 1992; Blanco et al., 1996; Smith et al., 2010). The diarrhoea calves associated with certain pathogenic *E. coli* may probably die or be related to primary infection with virus or mycoplasma if not be treated in time and often ended in maldevelopment even if survived (Matsuda et al., 2010). Therefore, for fear of significant economic losses, heavy amounts of antimicrobials are used in calves feed for preventive and curative purposes worldwide (Dheilly et al., 2011). As a result, the inevitable selection of antimicrobial resistance in calf pathogens and commensals may emerge and become a worldwide public health problem, including direct impact on food safety. This situation also led to the prevention and treatment failed because of antimicrobial resistance (Angulo et al., 2004; Okeke et al., 2005). Antimicrobial-resistant bacteria carried by animals can enter the human food chain through the consumption of meat or other animal products, through farm runoff water, and by other pathways (Donnelly, 1999). It has now become clear that antimicrobial resistance poses a threat to human and animal health and should be taken

*Corresponding author. E-mail: zxy0451@hotmail.com. Tel: 011-86-0451-55190674. Fax: 011-86-0451-55191200.
seriously (Blondeau and Vaughan, 2000; Mora et al., 2005).

Since E. coli, a reservoir of antimicrobial resistance genes, is considered as an excellent indicator of bacterial resistance at a population level investigating the prevalence of antimicrobial resistant isolates can facilitate risk assessment of infection and the choice of effective antimicrobial agents in clinical settings (Bogaard and Stobberingh, 2000).

Few data has been reported on antimicrobial resistant characterization of E. coli isolates from diarrheic calves although there are many studies about that of E. coli derived from other food animals (Lim et al., 2007; Smith et al., 2010; Li et al., 2010; Jiang et al., 2011), companion animals (Costa et al., 2008; Shaheen et al., 2010) and humans (Zhanel et al., 2006; Hoban et al., 2011). The aim of our present study was to investigate the prevalence of antimicrobial resistances in E. coli isolates recovered from diarrheic calves in Northeastern China and the mechanisms of resistance implicated in order to assess whether there is clonal dissemination of MDR strains among different animals in the same farm or among different farms.

MATERIALS AND METHODS

Bacterial strains

A total of 104 E. coli isolates were recovered from 357 rectal swabs of 2–10-week-old diseased calves as well as from the intestinal contents of dead calves from cases of post-weaning diarrhoea (P WD) from five different regions of Northeastern China (2008-2011). Each isolate was obtained from an individual calf which had no antibiotics application history. All bacteria samples were stored on ice and dispatched within 12 h to the Veterinary Medicine Academy of Northeast Agricultural University, and isolated and purified on MacConkey agar (TIAN HE Microorganism Reagent Co., Hangzhou, China) and identified using the API 20E kit (API System, Shanghai, China). Plasmid DNA for PCR was extracted by E.Z.N.A® Plasmid Miniprep Kit (Omega Bio-Tek). The PCR reaction mixture contained 2 µL of template DNA, 2.5 µL of 10X Ex Taq Buffer (Takara, Kyoto, Japan), 0.5 µL of each primer, and 2.5 µL of dNTP mixture (2.5 mM of each dNTP) in a final volume of 25µL. The PCR was done using an ABI 2720 thermal cycler (Applied biosystems, USA). Annealing temperature for each antimicrobial resistant gene is in Table 1. The amplified products were analyzed in 1.0% agarose gels by electrophoresis, and recorded with a gel documentation system. The reactions were run in duplicate to confirm results and representative amplification products were sequenced to verify PCR products. Where positive controls were not available, PCRs were carried out using described parameters, suspected positive amplicons were purified and sequenced. E. coli ATCC 25922 strains was used as negative controls.

Amplification and sequencing of gyrA, gyrB and parC genes

The quinolone resistance-determining region (QRDR) of the gyrase subunit A (gyrA), as well as the analogous region of the gyrase subunit B (gyrB) and topoisomerase IV subunit C (parC), present in all 104 E. coli isolates were amplified by PCR. The primers and their annealing temperature are listed in Table 1. The genomic DNA of the resistant E. coli isolates extracted by E.Z.N.A® Bacterial DNA Kit (Omega Bio-Tek) was used as the template of PCR. The DNA Sequences obtained were compared with those previously reported for gyrA (GenBank accession number X06373), gyrB (NC_000913) and parC genes (M58408 with the modification included in L22025).

Pulsed field gel electrophoresis (PFGE)

PFGE was used to analyze the genomic relatedness among E. coli isolates from diseased calves. We randomly selected 16 typical isolates with the same phenotypes of resistance (AMC: STR-TEM-CTX-M-1-SXT-DOX) from five different regions of northeastern China. PFGE of chromosomal DNA digested with the restriction enzyme XbaI was carried out according to a standard protocol using a CHEF-MAPPER System (Bio-Rad Laboratories, Hercules, CA, USA) as described by Gauton (1997). The gels were run at 6.0 V/cm with an angle of 120° at 14°C for 19 h. The Bio-rad 170-3605 CHEF DNA served as size marker.

RESULTS

Antimicrobial susceptibility of E. coli isolates from the diseased calves

The susceptibility to 14 antimicrobial agents for these 104
Table 1. Primer sequences used to amplify antimicrobial resistance genes and quinolone resistance-determining region.

| Antimicrobial family | Genes | Primer sequence (5’→3’) | Annealing (°C) | Fragment size (bp) | Reference |
|----------------------|-------|--------------------------|----------------|-------------------|-----------|
| **β-lactams**        | blaCTX-M-2 | GGCACCTGATCCCATGATGTTAATGTTAACATACAAATCC CGGTAGATTTGCCTTCCTGAAGGC | 55 | 351 | Pitout et al. (2004) |
|                      | blaTEM | GAGTATTCACATTTTCTG | 50 | 286 | Maynard et al. (2003) |
|                      | blaSHV | MCCAGTATTTACAGTGAC TCGCTGTGTTATATCTCCC | 50 | 698 | Maynard et al. (2003) |
|                      | blaOXA | GCCAGGAGCAGTCATCAAC | 50 | 888 | Maynard et al. (2003) |
|                      | aadA1 | TGGATTCTGTTAACTGCGTG | 58 | 284 | Van et al. (2008) |
|                      | aac(3)-II | ACTGTGATGGGATACGCGTC CTCCGTCAGCGTTTCAGCATAAC | 60 | 237 | Saenz et al. (2004) |
|                      | tetA | GTCGAACCACCAATACCC AGAACCAAGCAGGATGTAAGG | 50 | 887 | Van et al. (2008) |
|                      | tetB | CTTATCATGCGCACGTCGTGC GCGGCTGTTCCTGCC | 50 | 773 | Van et al. (2008) |
|                      | tetC | ACTTGGAGCAGCATCAGAC CTCAAATCCATGCAAACCC | 50 | 880 | Van et al. (2008) |
| **Aminoglycosides**  | sulI | TTCGGCATTCTGAATCTCAG | 50 | 822 | Maynard et al. (2003) |
|                      | sulII | ATGATCTAACCCTCGGTCTC CCGCATCGTCAACATAACC GTTGCGGATGAAAGTCAG | 50 | 722 | Saenz et al. (2004) |
|                      | trimethoprim | AAGAATGGAGTTATCGGGAATG | 50 | 391 | Maynard et al. (2003) |
|                      | dhfrI | GGTTAAAAACTGCGTAAAAATTTG CTGCAAAGCGAAAAACGG | 50 | 432 | Maynard et al. (2003) |
|                      | dhfrV | AGCAATAGTTAATGCTTGGAGCTAAG CAGGTGAGCAGAGATTTT | 50 | 294 | Maynard et al. (2003) |
|                      | dhfrXIII | CCTCAAAGGTTTGATGTCAG | 50 | 722 | Saenz et al. (2004) |
| **Tetracyclines**    | floR | CAGCTGTGACGCCTCTATAT ATGCAGAATGGAACGCGGG | 55 | 868 | Sáenz et al. (2004) |
|                      | cmlA | TGCTATTACCAGCATCTCG ATCGCGCATCCATTCAT | 55 | 455 | Sáenz et al. (2004) |
| **Sulphonamides**    | GyrA | TACACGGCAGCGATCATGTCGG TTAATTTAGGGCGCGTGGC | 64 | 647 | Bansal et al. (2011) |
|                      | GyrB | TCCTCCCAGACCAAAGAC | 64 | 447 | Bansal et al. (2011) |
|                      | ParC | TCACGACGATACCAACAGCC AAACCTGTTAGCAGCAGCGAT | 64 | 395 | Bansal et al. (2011) |
isolates is presented in Table 2. 100% of the isolates showed resistance to amoxicillin, streptomycin, tetracycline and sulfamethoxazole-trimethoprim, and 96.15% exhibited doxycycline resistance. The resistant rate to the other antimicrobial agents was in all cases below 19.23%, and no resistant isolates were detected to gentamicin, amikacin sulphate, neomycin and polymyxin b.

The phenotypes of resistance exhibited by 104 E. coli isolates are presented in Table 3. All the 104 isolates showed resistance to more than four antimicrobials and all of them were multidrug-resistant (MDR) isolates. Obviously, the most frequent detected phenotype was AMC- STR-TET- SXT- DOX which was found among 53.8% of these resistant isolates, followed by AMC- STR-TET- SXT- DOX-CTF (11.5%). The phenotypes of these E. coli isolates from the five regions showed highly similarity which could be easily found from the resistance to amoxicillin, streptomycin, tetracycline, doxycycline, sulfamethoxazole-trimethoprim and the susceptibility to gentamicin, amikacin sulphate, neomycin, polymyxin b. This reveals the same uses of antimicrobial agents of the nearby areas or there maybe clonal dissemination of identical resistant E. coli clones among farms nearby.

**Antimicrobial resistance genes and QRDR detected in E. coli isolates from diseased calves**

All the 104 isolates were screened for the presence of genes coding for 16 resistance determinants. Thirteen (13) out of the 16 resistance genes [blaTEM, bla

### Table 2. Antimicrobial resistance rate of E. coli isolated from different regions around Harbin.

| Antimicrobials | No. of isolates | Resistance | Intermediate | Susceptible |
|----------------|-----------------|------------|--------------|-------------|
| AMC            | 104             | 0          | 0            | 0           |
| CTF            | 20              | 0          | 0            | 84          |
| STR            | 104             | 0          | 0            | 0           |
| GM             | 0               | 0          | 0            | 104         |
| AMI            | 0               | 0          | 0            | 104         |
| NEO            | 0               | 0          | 0            | 104         |
| TET            | 104             | 0          | 0            | 0           |
| DOX            | 100             | 4          | 0            | 66          |
| CMP            | 20              | 58         | 0            | 26          |
| FLR            | 20              | 12         | 0            | 72          |
| P(B)           | 0               | 0          | 0            | 104         |
| SXT            | 104             | 0          | 0            | 0           |
| CIP            | 8               | 20         | 0            | 76          |
| NOR            | 12              | 22         | 0            | 70          |
| EN             | 4               | 14         | 0            | 86          |

AMC, Amoxicillin-clavulanic acid; CTF, ceftiofur; STR, streptomycin; GM, gentamicin; AN, amikacin; NEO, neomycin; TET, tetracycline; DOX, doxycycline; CMP, chloramphenicol; FLR, florfenicol; PB, polymyxin; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; NOR, ciprofloxacin; EN, enrofloxacin base.

### Table 3. Phenotypes of resistance detected among the 104 E. coli isolates from calves.

| Phenotype of resistance | No. of isolates | Percentage of isolates (%) |
|-------------------------|----------------|---------------------------|
| AMC-STR-TET- SXT        | 4              | 3.8                       |
| AMC- STR-TET- SXT- DOX  | 56             | 53.8                      |
| AMC- STR-TET- SXT- DOX-CTF| 12            | 11.5                      |
| AMC- STR-TET- SXT- DOX- NOR-CTF| 8           | 7.7                       |
| AMC- STR-TET- SXT- DOX- CMP-FLR| 8           | 7.7                       |
| AMC- STR-TET- SXT- CMP-FLR-CTF| 4            | 3.8                       |
| AMC- STR-TET- SXT- CMP-FLR- CIP| 4            | 3.8                       |
| AMC- STR-TET- SXT- CMP-FLR- CIP-EN| 4            | 3.8                       |
Table 4. Genes of resistance detected among 104 E. coli isolates of calf origins.

| Antibiotics   | Number of isolates | Genes detected | Number of isolates | Genes detected | Number of isolates |
|---------------|--------------------|----------------|--------------------|----------------|--------------------|
|               |                    | Resistant       | Susceptible and Intermediate | Resistant       | Susceptible and Intermediate |
| AMC           | 104                | blatem          | 80                 |                | —                  |
|               |                    | blatem+blactx-m | 8<sup>a</sup>      | 0              | —                  |
|               |                    | blactx-m-2      | 12<sup>a</sup>      | 0              | —                  |
| Streptomycin  | 104                | aadA1           | 76                 | 0              | —                  |
| Gentamicin    | 0                  | —               | —                  | 104            | aac(3)-II          |
| Tetracycline  | 104                | tetA            | 17<sup>b</sup>      | 0              | —                  |
|               |                    | tetB            | 65<sup>c</sup>      | 0              | —                  |
|               |                    | tetA+tetB       | 18<sup>c</sup>      | 0              | —                  |
| Chloramphenicol | 20                | cmlA+floR       | 20<sup>d</sup>      | 84             | cmlA               |
| SXT           | 104                | sulf            | 5                  | 0              | —                  |
|               |                    | sulf+dhfrV      | 49                 |                | —                  |
|               |                    | sulf+dhfrXIII   | 19                 |                | —                  |
|               |                    | sulf+dhfrI      | 4                  |                | —                  |
|               |                    | sulf+dhfrV      | 8                  |                | —                  |
|               |                    | sulf+dhfrXIII   | 17                 |                | —                  |

<sup>a</sup> These isolates also showed resistance to ceftiofur. <sup>b</sup> Thirteen of these isolates also showed resistance to doxycycline. <sup>c</sup> These isolates also showed resistance to doxycycline. <sup>d</sup> These isolates also showed resistance to florfenicol.

aadA1, aac(3)-II, tetA, tetB, cmlA, floR, sulf, sulfI, dhfrI, dhfrV and dhfrXIII] were detected. All 104 isolates carried one or more antimicrobial resistance genes evaluated. The resistance genotypes for beta-lactams, aminoglycosides, tetracyclines, amphenicols, sulphonamides and trimethoprim in all the 104 E. coli isolates are presented in Table 4.

Beta-lactam resistance gene blatem was found in 88 (86.4%) isolates out of 104 AMC-resistant isolates. 20 (19.2%) isolates (8 blatem positive isolates and 12 blatem negative isolates) showed ceftiofur resistance and all of them harboured the resistance gene blactx-m-2 (Table 4). No beta-lactamases genes were identified in the remaining four AMC-resistant isolates. Other beta-lactam resistance genes (blaOXA and blaSHV) were not detected.

Of 104 streptomycin-resistant E. coli isolates, 76 (73.3%) isolates harboured aadA1 gene. However, it seems paradoxical that aac(3)-II was found in 12 (11.5%) isolates with none of the isolates showed resistance to gentamicin (Table 4). In addition, 20 isolates showed resistance to both chloramphenicol and florfenicol. The floR gene was amplified from these 20 (19.2%) isolates and the cmlA gene was amplified from these 20 isolates and other 28 susceptible isolates (Table 4). Tetracycline resistance genes [tetA and/or tet(B) genes] were found in all 104 tetracycline-resistant. The tetB gene was most common, found in 83 (79.8%) isolates, and the tetA gene in 35 (33.7%) isolates. Fourteen (14) isolates carried two (tetA and tetB) genes (Table 4). TetC was not detected in any isolates.

Among 104 SXT-resistant E. coli isolates, the sulfonamide efflux resistance gene sulf was found in 77 (74.0%) isolates and sulfI found in only 25 (24.0%). Three common trimethoprim resistance (dhfr) genes were targeted: dhfrI, dhfrV and dhfrXIII. The dhfrV and dhfrXIII genes were observed in 57 (54.8%) and 36 (34.6%) isolates, whereas dhfrI was only found in 4 (3.8%) isolates (Table 4). But five SXT-resistant isolates did not carry any of dhfr genes and two isolates did not carry either sulfonamide efflux resistance genes or dhfr genes evaluated. They might carry other or even novel genetic resistance determinants.

The gyrA, gyrB and parC genes were amplified and sequenced in all 104 isolates and the deduced amino acid changes detected in GyrA and ParC proteins are shown in Table 5. One amino acid changes in GyrA
Table 5. Amino acid changes in GyrA and ParC proteins deduced from the sequences of the corresponding genes in our quinolone-resistant E. coli isolatesa.

| Phenotype of resistance to quinolonesa | Number of E. coli isolates | Amino acid changes in GyrA | Amino acid changes in ParC |
|--------------------------------------|---------------------------|---------------------------|---------------------------|
| Norfloxacin                          | 2                         | Ser83Leu                  | wild                      |
| Norfloxacin                          | 10                        | wild                      | Ser80Arg                  |
| Ciprofloxacin                        | 4                         | wild                      | Ser80Ile                  |
| Ciprofloxacin-enrofloxacin base      | 2                         | Ser83Leu                  | wild                      |
| Ciprofloxacin-enrofloxacin base      | 2                         | wild                      | Ser80Arg                  |

*aSequences were compared with gyrA and parC genes included in the GenBank database with the accession numbers X06373 for gyrA and M58408 with the modification in L22025 for parC.

(Ser83Leu) in two norfloxacin-resistant isolates and one in ParC (Ser80Arg) in other ten norfloxacin-resistant isolates found in this study. Only one amino acid change in ParC (Ser83Ile) was found in the four ciprofloxacin-resistant isolates. And one amino acid changes in GyrA (Ser83Leu) in two ciprofloxacin-enrofloxacin base-resistant isolates and one in ParC (Ser80Arg) in the other two ciprofloxacin-enrofloxacin base-resistant isolates. No mutation was detected in gyrB in this study.

Pulsed field gel electrophoresis (PFGE)

To investigate the possibility of clonal spreading, a total of 16 isolates with the same phenotype of resistance from five different geographical regions were analyzed by PFGE. The resulting dendrogram shows diverse group clusters in XbaI-digested PFGE patterns of isolates (Figure 1). Isolates D14 and H2 had closely related banding patterns, and isolates D2, H6, Z16 and Z20 had closely related PFGE profiles. Isolates S11, S12 and A3 had closely related banding patterns, and isolates S8 and H9 had closely related PFGE profiles. Genomic typing demonstrated that at least four closely related M. catarrhalis clones had been transmitted in five regions of northeastern China.

DISCUSSION

It is noteworthy that our study revealed a very high incidence rate of resistance for amoxicillin (100%),
among our isolates from five farms. These findings were similar with previous studies reporting that TEM β-lactamases were the most frequent mechanism in ampicillin-resistant *E. coli* isolates from food-producing animals (Mora et al., 2005; Lim et al., 2007; Jiang et al., 2011; Ryu et al., 2012). Our findings have provided another confirmation that TEM β-lactamase was the main mechanism of amoxicillin resistance.

In our 104 streptomycin-resistant isolates, aminoglycoside resistant gene aadA1 was found in 76 (73.3%) isolates, which was similar to some previous studies (Chang et al., 2007; Costa et al., 2008; Ryu et al., 2012). In cases where the gene aadA1 was not identified, streptomycin resistance may be due to other resistance gene (strA or strB for instance) that was not screened in the present study. Four classes of aac(3) acetyltransferases have been reported associated with gentamicin resistance in *E. coli* (Costa et al., 2008). In this study, aac(3)-II was found in 12 (11.54%) isolates with none of the isolates showed gentamicin resistance. Nevertheless, the similar phenomenon had been previously reported (Chen et al., 2005). From these results, we can see that it is possible that resistance genes may not be expressed or expressed in a low level in actuality (Meacham et al., 2003; Ryu et al., 2012). Therefore, the search for antimicrobial resistance genes should not only be limited to phenotypically resistant isolates (Ryu et al., 2012).

In our study, 20 isolates showed chloramphenicol-florfenicol cross-resistance and chloramphenicol resistance gene cmlA and florfenicol resistance gene floR were both amplified in the 20 isolates. There is a previous report about *E. coli* isolates from calf harbouring both floR and the cmlA genes (Du et al., 2004). Since the cmlA gene shares 50.4% homology with floR gene, it is very possible that at some point they may have evolved from a common ancestor resistance gene (Douillet et al., 2002). And in our study, isolates contained gene floR and gene cmlA showed resistance to chloramphenicol while isolates only contained gene cmlA showed resistance to chloramphenicol. So we could imagine that the main mechanism of chloramphenicol resistance was gene floR which was verified chloramphenicol-florfenicol cross-resistance gene and could developed majority of chloramphenicol resistance in calf isolates (Dolejska´ et al., 2008).

The detection of tet(A) and/or tet(B) genes in all our tetracycline-resistant isolates indicates that the main mechanism of tetracycline resistance in calf *E. coli* isolates is by active efflux. A predominance of tet(B) gene has been observed among tetracycline-doxycycline-resistant *E. coli* isolates of diarrheic calves in this study which was also reported in clinical isolates of calves by other authors from different countries (Dolejska’ et al., 2008).

The high percentage of resistance to SXT correlates with the high prevalence of genes of antibiotic resistance
to both antibiotics. Such high levels of resistance to this combined antibiotic in E. coli isolates from calf had never been reported previously. One given explanation was their widespread use in the treatment of diseases associated with Gram-negative bacteria, especially with acute infectious diarrhoea. And the majority of sul and dhfr genes are related to mobile elements of antibiotic resistance such as integron, which would be consistent with antibiotic exposure explaining their high prevalence in the study population (Costa et al., 2008; Smith et al., 2010; Soufi et al., 2011).

Four amino acid changes in gyrA (4Ser83→Leu) and sixteen in parC (twelve Ser80→Arg, four Ser80→Ile) were identified in the 20 fluoroquinolone-resistant E. coli isolates in this study. No mutation was detected in gyrB. This observation was in agreement with previous reports that mutations in gyrA and parC were common among fluoroquinolone-resistant strains of E. coli and Salmonella, whereas mutations in gyrB were rare (Bansal et al., 2011; Yang et al., 2012).

Overall, when the E. coli obtained the resistance, a corresponding resistance gene may be detected most of the time. In cases where the gene was not identified, resistance may be encoded by other resistance gene that was not screened in the present study or due to other mechanisms of resistance. Another situation should be noted that the resistant genes were detected not only from the resistant isolates but also from the susceptible isolates. The reason was complex and the most credible explanation was that the genes may not be expressed. Therefore, investigating antimicrobial resistance genes should not only be limited to phenotypically resistant isolates, but also to susceptible isolates. So far, some studies reported that antimicrobial resistance genes have been detected in some susceptible E. coli isolates (Lanz et al., 2003; Srinivasan et al., 2007; Ryu et al., 2012).

In this study, PFGE was performed for subtyping the isolates to determine the genetic relatedness of isolates with the same resistance phenotype. Although the selected number was small (16/104, 15.4%), we found four closely related clones in five different geographical regions, which indicates that these clones are spreading in Northeastern China. Preventive measures should be adopted to control the clonal dissemination of MDR strains.

According to our knowledge, this is the first report about prevalence of antimicrobial resistance and resistance genes in E. coli from diarrheic calves in northeastern China. All the 104 E. coli isolates showed multi-drug resistance, and very high percentages of resistance to amoxicillin, streptomycin, tetracycline, doxycycline and sulfamethoxazole-trimethoprim have been detected, which were much higher than those reported in other countries. Observations from this study do not exactly explain the link between antimicrobial usage and an increase of antimicrobial resistance among E. coli isolates. However, our observations have suggested that clonal dissemination of MDR strains among different animals in the same farm or among different farms played an important role in the severe antimicrobial resistance in northeastern China and our observations have provided data to display the prevalence of 13 kinds of resistant genes in northeastern China.

ACKNOWLEDGEMENTS

The research work was supported by The State Key Laboratory of Veterinary Biotechnology Open Fund (SKLVBF201107). The authors are grateful to the Director, Harbin Veterinary Research Institute (Heilongjiang, China) for allowing use of the facilities for PFGE and Dr. Xiaoyan Yu (Chinese center for disease control and prevention, Beijing, China) for her assistance with the statistical analysis.

Abbreviations

15 antibiotics

AMC, amoxicillin-clavulanic acid; CTF, ceftiofur; STR, streptomycin; GM, gentamicin; AMI, amikacin sulphate; NEO, neomycin; NOR, norfloxacin; CIP, ciprofloxacin; EN, enrofloxacin base; TET, tetracycline; DOX, doxycycline; CMP, chloramphenicol; FLR, florfenicol; SXT, sulfamethoxazole-trimethoprim; P(B), polymyxin b.; bla TEM, beta-lactamase TEM; bla CTX-M-2, beta-lactamase CTX-M-2; bla SHV, beta-lactamase SHV; bla OXA, beta-lactamase OXA; aadA1, aminoglycoside adenyllyltransferase A1; aac(3)-II, aminoglycoside N(3')-acetyltransferase II; flo R, florfenicol resistant gene; cmlA, chloramphenicol acetyltransferase 2; tetA, tetracycline resistance genes A; tet B, tetracycline resistance genes B; tet C, tetracycline efflux resistance genes C; sul I, sulfonamide resistance gene I; sul II, sulfonamide efflux resistance gene II; dhfr I, trimethoprim resistance gene I; dhfr V, trimethoprim resistance gene V; dhfr XIII, trimethoprim resistance gene XIII; gyr A, DNA gyrase (type II topoisomerase), subunit A; gyr B, DNA gyrase (type II topoisomerase), subunit B; Ser, serine parC: DNA topoisomerase IV, subunit B; Leu, leucine; Arg, Arginine; MDR, multi-drug resistance; ARGs, antibiotics resistance genes; QRDR, quinolone resistance-determining region; CLSI, Clinical and Laboratory Standards Institute; PFGE, Pulsed field gel electrophoresis.

REFERENCES

Angulo FJ, Baker NL, Olsen SJ, Anderson A, Barrett TJ (2004). Antimicrobial use in agriculture: controlling the transfer of antimicrobial resistance to humans. Semin. Pediatr. Infect. Dis. 15:78-85.
Bansal S, Tandon V (2011). Contribution of mutations in DNA gyrase and topoisomerase IV genes to ciprofloxacin resistance in Escherichia coli clinical isolates. Int. J. Antimicrob. Agents 37:253-255.

Blanco J, Cid D, Blanco JE, Blanco M, Quitteira JR, Fuente R (1996). Serogroups, toxins and antibiotic resistance of Escherichia coli strains isolated from diarrheic lambs in Spain. Vet. Microbiol. 49:209-217.

Blondeau JM, Vaughan D (2000). In vitro activity of 19 antimicrobial agents against 3513 nosocomial pathogens collected from 48 Canadian medical centres. The Canadian Antimicrobial Study Group. Int. J. Antimicrob. Agents 15:213-215.

Bogaard AE, Stobberingh EE (2000). Epidemiology of resistance to antibiotics: Links between animals and humans. Int. J. Antimicrob. Agents 14:327-335.

Chang LL, Chang TY, Chang CY (2007). Variable Gene Cassette Patterns of Class 1 Integron-Associated Drug-Resistant Escherichia coli in Taiwan. Kaohsiung J. Med. Sci. 23:273-280.

Chen S, Zhao S, McDermott PF, Schroeder CM, White DG, Meng J (2005). A DNA microarray for identification of virulence and antimicrobial resistance genes in Salmonella serovars and Escherichia coli. Mol. Cell. Probe 19:195-201.

Costa D, Poeta P, Sáenz Y, Coelho AC, Sáenz Y, Briñas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, Torres C (2007). Variable Gene Resistance Determinants in Clinical Escherichia coli isolates. Vet. Microbiol. 149:422-429.

Dolejska A, Senk D, Cizek A, Rybankova J, Syricha O, Litara K (2008). Antimicrobial resistant Escherichia coli isolates in cattle and house sparrows on two Czech dairy farms. Res. Vet. Sci. 85:491-494.

Donnelly JP (1999). Commentary on the MAFF technical report: a review of antimicrobial resistance in the food chain. Int. J. Antimicrob. Agents. Agent. 12:63-65.

Doubelt B, Schwarz S, Nebbekke E, Baucheron S, Martel JC, Danclea EC, Cloeckaerta A (2002). Molecular analysis of chromosomally and plasmid-mediated resistance to antimicrobial agents in Escherichia coli isolates from France and Germany. J. Antimicrob. Chemother. 49:49-54.

Du XD, Xu C, Shen JZ, Wu BB, Shen ZQ (2004). Characterization of florfenicol resistance among calf pathogenic Escherichia coli. FEMS Microbiol. Lett. 236:183-189.

Hoban DJ, Nicolle LE, Hawser S, Bouchillon S, Badal R (2011). Antimicrobial susceptibility of global inpatient urinary tract isolates of Escherichia coli: results from the Study for the Monitoring Antimicrobial Resistance Trends (SMART) program: 2009–2010. Diagn. Microbiol. Infect. Dis. 70:507-511.

Jiang HX, Li DH, Chen ZL, Wang XM, Chen JR, Liu YH, Liao XP, Liu JH, Zeng ZL (2011). High prevalence and widespread distribution of multi-resistant Escherichia coli isolates in pigs and poultry in China. Vet. J. 187:99-103.

Lanz R, Kuhnert P, Boerlin P (2003). Antimicrobial resistance and resistance gene determinants in clinical Escherichia coli from different animal species in Switzerland. Vet. Microbiol. 91:79-84.

Li L, Jiang ZG, Xie LN, Shen JZ, Dai L, Wang Y, Huang SY, Wu CM (2010). Characterization of antimicrobial resistance and molecular determinants of beta-lactamase in Escherichia coli isolated from chickens in China during 1970–2007. Vet. Microbiol. 144:505-510.

Lim SK, Lee HS, Nam HM, Cho YS, Kim JM, Song SW, Park YH, Jung SC (2007). Antimicrobial resistance observed in Escherichia coli strains isolated from fecal samples of cattle and pigs in Korea during 2003–2004. Int. J. Food. Microbiol. 116:283-286.

Matsuda K, Chaudhari AA, Lee JH (2010). Avian colibacillosis caused by an intestinal pathogenic Escherichia coli isolate from cull diarrhea. Res. Vet. Sci. 89:150-152.

Maynard C, Fairbrother JM, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, Lariviere S, Harel J (2003). Antimicrobial resistance genes in enterotoxigenic Escherichia coli O157:H7 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Ch. 47:3214–3221.

Meacham KJ, Zhang L, Foxman B, Bauer RJ, Marrs CF (2003). Evaluation of geno-typing large numbers of Escherichia coli isolates by entero bacterial repetitive intergenic consensus-PCR. J. Clin. Microbiol. 41:5224-5226.

Mora A, Blanco JE, Blanco M, Alonso MP, Dhaba G, Cheeta A, González EA, Bernárdez M, Nysalusu JB, Murray J, Perovic O, Koornhof H (2005). Antimicrobial resistance of Shiga toxin (verotoxin)-producing Escherichia coli O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol. 156:793-806.

Okeke IN, Klugman KP, Bhuta ZA, Duse AG, Jenkins P, O'Brien TF, Mendez AP, Laxminarayan R (2005). Antimicrobial resistance in developing countries. Part II: strategies for containment. Lancet Infect. Dis. 5:568-580.

Pitout JD, Hossain A, Hanson ND (2004). Phenotypic and molecular detection of CTX-M-8-lactamases produced by Escherichia coli and Klebsiella spp. J. Antimicrob. Chemother. 42:5715-5721.

Rybaková J, Dolejská M, Materna D, Litářík I, Cizek A (2010). Phenotypic and genotypic characteristics of antimicrobial resistant Escherichia coli isolated from symbionves flies, cattle and sympatric insectivorous house martins from a farm in the Czech Republic (2006–2007). Res. Vet. Sci. 89:179-183.

Ryu SH, Park SG, Choi SM, Hwang YO, Han HJ, Kim SU, Lee YK, Kim MS, Park KY, Kim KS, Chae YZ (2012). Antimicrobial resistance and resistance genes in Escherichia coli strains isolated from commercial fish and seafood. Int. J. Food. Microbiol. 150:14-18.

Sáenz Y, Briñas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, Torres C (2004). Mechanisms of resistance in multiple-antibiotic-resistant Escherichia coli strains of human, animal, and food origins. Antimicrob. Agents Ch. 48:3996-4001.

Shaheen BW, Oyarzabal OA, Boothe DM (2010). The role of class 1 and 2 integrons in mediating antimicrobial resistance among canine and feline clinical E. coli isolates from the US. Vet. Microbiol. 144:363-370.

Smith MG, Jordan D, Chapman TA, Chin JJ, Barton MD, Do TN, Fahy VA, Fairbrother JM, Trott DJ (2010). Antimicrobial resistance and virulence gene profiles in multi-drug resistant enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhea. Vet. Microbiol. 145:299-307.

Souti L, Sáenz Y, Vinué L, Abbassi MS, Ruiz E, Zarazaga M, Hassen AB, Hammami S, Torres C (2011). Escherichia coli of poultry food origin as reservoir of sulphonamide resistance genes and integrons. Int. J. Food. Microbiol. 144:497-502.

Srinivasan V, Gillespie BE, Lewis MJ, Nguyen LT, Headrick SI, Schukken YH, Stephen P (2007). Oliver Phenotypic and genotypic antimicrobial resistance patterns of Escherichia coli isolated from dairy cows with mastitis. Vet. Microbiol. 124:14-18.

Van TT, Chin J, Chapman T, Tran LT, Coloe PJ (2008). Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes. Int. J. Food. Microbiol. 124:217-223.

Vihan VS, Kala SN, Singh VP (1992). Epidemiological investigation of neonatal kidney mortality due to enteropathogenic colibacillosis. Prev. Vet. Med. 13:179-183.

Yang BW, Xi ML, Cui SH, Zhang XL, Shen JL, Sheng M, Qu D, Wang X, Meng JH (2012). Mutations in gyrase and topoisomerase genes associated with fluoroquinolone resistance in Salmonella serovars from retail meats. Food Res. Int. 45:935-939.

You JY, Moon BM, Oh IG, Baek BK, Li LG, Kim BS, Stein BD, Lee JH (2006). Antimicrobial resistance of Escherichia coli O157 from cattle in Korea. Int. J. Food Microbiol. 106:74-78.

Zhang SG, Hisanaga TM, Laing NM, DeCorby MR, Nichol KA, Piatnik LP, Johnson J, Noreddin A, Harding GK, Nicolle LE, Hoban DJ, NAUTICA Group (2006). Antibiotic resistance in Escherichia coli outpatients’ urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int. J. Antimicrob. Agents 27:468-475.