Escherichia coli is one of the important members of the Escherichia genus. Escherichia coli of some specific serotypes are pathogenic to animals and cause severe diarrhea, fowl cellulitis, swollen head syndrome, peritonitis, salpingitis, synovitis, umbilical cord inflammation, ophthalmia, fibropericarditis, and septicemia (Cai, 2001). The lower productivity and death of chicks caused by E. coli lead to severe economic losses throughout the world.

Sulfonamide antibiotics have such advantages as low price, convenient storage, broad antimicrobial spectrum, and remarkable efficacy. At present, they are still essential drugs for the prevention and control of bacterial diseases. As a result of long-term high-dose application, resistance to sulfonamide antibiotics has become serious, as demonstrated by previous studies (Yang et al., 2007; Zhou et al., 2007). Currently, the resistance mechanisms for sulfonamide antibiotics in E. coli have been clarified. Sulfonamide resistance (SulR) genes cause high-level expression of dihydropteroate synthase that mediates sulfonamide resistance. These genes are transferred between commensal E. coli via integrons, transposons, or plasmids (Hammerum et al., 2006), leading to broad drug resistance. The SulR genes include Sul1, Sul2, and Sul3 genes. Among them, Sul2 gene is the most widely distributed SulR gene in porcine, avian, or human E. coli, and it plays an important role in sulfonamide resistance (Blahna et al., 2006). Many experiments have been conducted to study sulfonamide antibiotic-resistant phenotypes of E. coli (Shen and Wu, 2007; Lai et al., 2011). However, regional distribution of SulR genes is rarely reported. To observe the distribution of SulR genes in North China and to guide drug application for avian colibacillosis, we monitored resistance to sulfonamide antibiotics in avian E. coli and detected SulR genes by PCR.

**MATERIALS AND METHODS**

**Isolation of E. coli**

Avian E. coli isolates were isolated from 28 large-scale chicken farms in Beijing, Tianjin, Inner Mongolia, Shanxi, and Hebei regions of China. Livers, pericardial fluid, feces, and other pathological materials were collected from dead chickens under sterile conditions, cross-inoculated in MacConkey medium (Tianhe Microorganism Reagent Co. Ltd., Hangzhou, China), and single pink colonies were picked and purified on MacConkey medium. In total, 164 isolates were identified as E. coli by biochemical tests.
Reagents

Drug-sensitive paper discs respectively saturated with sulfamonomethoxine (SMM) and sulfamethoxazole-trimethoprim (SXT) were purchased from Hangzhou Tianhe Microorganism Reagent Co. Ltd. Taq DNA polymerase, PCR kits, DNA Markers, dNTPs, agarose, and other reagents were purchased from TaKaRa (Dalian, China).

Drug Susceptibility Test

Drug susceptibility was detected by the Kirby-Bauer disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2003). Bacterial liquid was uniformly spread onto the agar medium (Tianhe Microorganism Reagent Co. Ltd.), and drug-sensitive slips were placed with at least 30-mm intervals on the medium. Then, the inhibition zone diameters were examined after incubation for 18 to approximately 24 h at 37°C. Drug resistance was identified using the standard proposed by the Clinical and Laboratory Standards Institute (CLSI, 2003).

PCR Amplification of Sulfonamide Resistance Genes

Primers, shown in Table 1, were designed as described previously (Zhou et al., 2007) and synthesized by Beijing Sunbiotech Co. Ltd. (Beijing, China). Bacterial chromosomal DNA was extracted routinely by the alkaline lysis method (Sambrook and Russell, 2008) and dissolved in 40.0 μL of TE buffer (pH 8.0) for use. Following the manufacturer’s instructions, PCR was performed in a MyCycler thermal cycler (Bio-Rad, Hercules, CA). The PCR reaction mixture was comprised of 5.0 μL of 10× Taq buffer, 2.5 μL of chromosomal DNA, 0.8 mM/L of dNTPs, 0.2 μM/L of each primer, 1.25 units of Taq DNA polymerase, and made up to a final volume of 50.0 μL with ultra-pure water. The temperature profile was as follows: 94°C, 5 min; (94°C, 50 s; 55°C, 45 s; 72°C, 50 s) × 32 cycles; 72°C, 6 min. The PCR products were preserved at 4°C.

Gel Electrophoresis and Sequencing

The mixture composed of 5.0 μL of PCR products and 1.0 μL of 6× loading buffer was subjected to electrophoresis at 80 V in 1.5% (wt/vol) agarose gels for 50 min using a JY1000C universal electrophoresis power supply (Beijing Liuqi Instrument Factory, Beijing, China). After electrophoresis, the gels were observed in a WD-9413C UV gel imaging system purchased from Beijing Liuqi Instrument Factory. The PCR products were sequenced by Beijing Sunbiotech Co. Ltd. (Beijing, China), and the obtained sequences were aligned using DNAStar suite (http://www.dnastar.com/).

RESULTS

Resistance to Sulfonamide Antibiotics in Avian E. coli Isolates

The avian E. coli isolates had obvious resistance to SMM and SXT, as evidenced by the drug sensitivity test. The rate of resistance to SMM was slightly higher than that to SXT, but the opposite result was obtained for the rate of sensitivity (Table 2).

Distribution of SulR Genes in Avian E. coli Isolates

The SulR genes were found in 90.85% (149/164) of the isolates, as evidenced by the results of PCR. Moreover, the Sul1 gene had the highest positive rate, up to 72.56% (119/164), followed by the Sul2 [62.80%...

Table 1. Characteristics of primer pairs specific for sulfonamide resistance (SulR) gene

<table>
<thead>
<tr>
<th>SulR gene</th>
<th>Primer sequence (5′→3′)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sul1</td>
<td>Forward: CAT TGC CTG GTT GCT TCA T</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>Reverse: ATC CGA CTC CCA GCA TTT</td>
<td></td>
</tr>
<tr>
<td>Sul2</td>
<td>Forward: CAT CAT TTT CGG CAT CGT C</td>
<td>793</td>
</tr>
<tr>
<td></td>
<td>Reverse: TCT TGC GGT TTC TTT CAG C</td>
<td></td>
</tr>
<tr>
<td>Sul3</td>
<td>Forward: AGA TGT GAT TGA TTT GGG AGC</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>Reverse: TAG TTG TTT CTG GAT TAG AGC CT</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial resistance against sulfamonomethoxine (SMM) and sulfamethoxazole-trimethoprim (SXT) in 164 avian Escherichia coli isolates

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance rate (%)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>SMM</td>
<td>90.24 (148/164)</td>
</tr>
<tr>
<td>SXT</td>
<td>73.17 (120/164)</td>
</tr>
</tbody>
</table>

¹The disc diffusion standard for drug resistance against antibiotics recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was used to evaluate drug resistance of the isolates based on the size of the inhibition zone.
(103/164) and Sul3 genes [49.39% (81/164)]. The positive rates include one gene appearing alone and in combination with the other two.

**Distribution of Multiple Sulfonamide Resistance Genes in Avian *E. coli* Isolates**

Most of the *E. coli* isolates carried multiple sulfonamide resistance genes (Table 3). Of the 164 isolates, 32 (accounting for 19.51%) had 3 commensal resistance genes. Two resistance genes were commensal in 54.88% (90/164) of the isolates. In addition, 16.46% (27/164) of the isolates carried a single *SulR* gene, and 66.67% (103/164) had a combination with the other two.

**Sequence Analysis of *SulR* Genes**

The amplified *SulR* genes had higher than 98% nucleotide sequence similarity to the corresponding sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/index.html) [ID: GQ293501 (*Sul1* gene), AY360321 (*Sul2* gene), and AY494779 (*Sul3* gene)], as analyzed using the DNAStar software. The similarities for the *Sul1*, *Sul2*, and *Sul3* genes were 98.3, 99.5, and 99.1%, respectively.

**DISCUSSION**

In this study, the rates of resistance to SMM and SXT were higher than 70% in the avian *E. coli* isolates, indicating serious sulfonamide resistance in North China. The results are consistent with rates of resistance to neazolin and sulfamethoxazole (89.55 and 86.57%, respectively) in *E. coli* in piglets, as surveyed in some regions of the Jilin Province of China in 2007 (Shen and Wu, 2007). However, the rate of resistance to sulfonamide antibiotics was 26.1% in *E. coli* isolated from some large-scale pig farms in Sichuan Province in 1999 (Wang et al., 1999). Therefore, the rate of resistance to sulfonamide antibiotics shows a remarkable increasing trend. The increase may be caused by long-term unreasonable use of sulfonamide antibiotics in poultry, which induces wide resistance in intestinal *E. coli*. Additionally, *SulR* genes mainly located on plasmids are resident on integron gene cassettes, and they can be easily transferred between bacteria via integrons, transposons, or plasmids (Bean et al., 2005; Liu, 2009).

In our study, the total positive rate of *SulR* genes (90.85%) was almost equal to the rate of resistance to SMM (90.24%) and SXT (73.17%). Thus, the positive rate of phenotype was basically in line with that of genotype for sulfonamide resistance. The results indicate that the 3 *SulR* genes are the most important resistance genes related to sulfonamide antibiotics. Particularly, no *SulR* gene was detected in some isolates that were resistant to SMM or SXT in the drug sensitivity test, possibly because such isolates contain other resistance genes or because other resistance mechanisms are involved.

The positive rates of the *Sul1* and *Sul3* genes in our study were basically the same as those in *E. coli* isolated from human manure, pig manure, and pork (Hammerum et al., 2006). We found that the *Sul1* gene had the highest positive rate among the 3 *SulR* genes. But many studies reported that the *Sul2* gene was the most widely distributed resistance gene in *E. coli* (Infante et al., 2005; Blahna et al., 2006; Chen et al., 2008). Our results show that the positive rate of the *Sul1* gene has increased in sulfonamide antibiotic-resistant bacteria, and sulfonamide resistance is mainly contributed by this gene in avian *E. coli* isolates in North China. Our investigation on the relationship between drug resistance and resistance phenotype can guide clinical medication for avian colibacillosis in North China and provide data for studies on resistance-gene transfer between bacteria.

**ACKNOWLEDGMENTS**

This work was supported by Hebei Key Technology R&D Program grant (10220414) from the Department of Science & Technology, Hebei Province, China.

**REFERENCES**


