Study on the association between drug-resistance and gene mutations of the active efflux pump acrAB-tolC gene and its regulatory genes

QUAN-PING MA1*, LIANG SU2,3*, JING-WEN LIU2,3, MING-XIAO YAO4 and GUANG-YING YUAN2,3

1Department of Clinical Laboratory, Jinan Fourth People's Hospital, Jinan, Shandong 250031;
2Department of Clinical Laboratory, Jinan Infectious Disease Hospital Affiliated to Shandong University, Jinan, Shandong 250021; 3Collaborative Innovation Center for The Origin and Control of Emerging Infectious Diseases, Taishan Medical University, Taian, Shandong 271016; 4Bacteria Laboratory, Shandong Center for Disease Control and Prevention, Shandong 250014, P.R. China

Received July 20, 2016; Accepted December 22, 2017

DOI: 10.3892/mmr.2018.8916

Abstract. The aim of the present study was to investigate the correlation between the multi-drug resistance of Shigella flexneri and the drug-resistant gene cassette carried by integrons; in the meanwhile, to detect the associations between drug-resistance and gene mutations of the active efflux pump acrAB-tolC gene and its regulatory genes, including marOR, acrA, acrB and soxS. A total of 158 isolates were isolated from the stool samples of 1,026 children with diarrhea aged 14 years old between May 2012 and October 2015 in Henan. The K-B method was applied for the determination of drug resistance of Shigella flexneri, and polymerase chain reaction amplification was used for class 1, 2 and 3 integrase genes. Enzyme digestion and sequence analysis were performed for the variable regions of positive strains. Based on the drug sensitivity assessment, multi-drug resistant strains that were resistant to five or more antibiotics, and sensitive strains were selected for amplification. Their active efflux pump genes, acrA and acrB, and regulatory genes, marOR, acrA and soxS, were selected for sequencing. The results revealed that 91.1% of the 158 strains were multi-resistant to ampicillin, chloramphenicol, tetracycline and streptomycin, and 69.6% of the strains were multi-resistant to sulfamethoxazole/trimethoprim. The resistance to cefazidime, ciprofloxacin and levofloxacin was <32.9%. All strains (100%) were sensitive to ampicillin, chloramphenicol, tetracycline and streptomycin, and 69.6% of the strains were multi-resistant to sulfamethoxazole/trimethoprim. The resistance to cefazidime, ciprofloxacin and levofloxacin was <32.9%. All strains (100%) were sensitive to ampicillin, chloramphenicol, tetracycline and streptomycin.

of the class 1 integron positivity was 91.9% (144/158). Among these class 1 integron-positive strains, 18 strains exhibited the resistance gene cassette dfrV in the variable region of the strain, four strains exhibited dfrA17-aadA5 in the variable region and 140 strains exhibited blaOXA-30-aadA1 in the variable region. Four strains showed no resistance gene in the variable regions. The rate of class 2 integron positivity was 86.1% (136/158), and all positive strains harboured the dfrA1-sat1-aadA resistance gene cassette in the variable region. The class 3 integrase gene was not detected in these strains. The gene sequencing showed the deletion of base CATT in the 36, 37, 38, 39 site in all positive strains harboured the dfrA1-sat1-aadA resistance gene cassette in the variable region. The class 3 integrase gene was not detected in these strains. The gene sequencing showed the deletion of base CATT in the 36, 37, 38, 39 site in

Introduction

Shigella is a genus of pathogens responsible for acute bacterial diarrhea, chronic bacteritic dysentery and toxic dysentery. Certain Shigella strains can cause hemolytic uremic syndrome and Reiter's chronic arthritis syndrome, and with the extensive use of antibacterial agents, bacterial drug-resistance is becoming a serious concern. Mobile genetic elements, including plasmids, transposons and integrons are important in the spread of antibiotic resistance (1). Among these elements, class 1, 2 and 3 integrons are associated with drug resistance. A previous study showed that the involvement of the active efflux pump is significant in bacterial multi-drug resistance (2). Previous studies have mainly focused on the active efflux pump of Escherichia coli, AcrAB-TolC, which is also expressed in Shigella strains (3). The regulation of gene mutation may beimportant in the multi-drug resistance mediated by the gene (4). In the present study, the characteristics of integron genes were analyzed in clinical Shigella isolates from children, mutations of the active efflux pump gene (acrAB-tolC) and regulatory genes were examined, and the association between these genes and the drug-resistance of Shigella was investigated. This may provide evidence for the
clinical treatment of dysentery and the control of multi-drug resistance of *Shigella*.

**Materials and methods**

**Location of isolated strains.** A total of 158 isolates of *Shigella flexneri* were isolated from the stool samples of 94 male cases and 64 female cases aged from 6 months to 14 years old between May 2012 and October 2015, in which there were 106 cases aged less than 5 years old. The isolates were identified using ID32E identification strips and an ATB expression instrument, and were serotyped using diagnostic-serum of *Shigella*. *Escherichia coli* (cat. no. 25922; American Type Culture Collection) was preserved in the Clinical Microbiology Laboratory of The Fourth Hospital of Jinan City (Jinan, China) and it was the quality control strain for antimicrobial susceptibility assessment. The present study was approved by the Ethics Committee of Jinan Infectious Disease Hospital Affiliated To Shandong University (Shandong, China). Written informed consent was obtained from all patients or patient guardians for the present study.

**Reagents and equipment.** The following were obtained for use in the present study: *Shigella* diagnostic serum (Lanzhou Institute of Biological Products Co. Ltd., Lanzhou, China); antibiotic discs of ampicillin, chloramphenicol, tetracycline, streptomycin, trimethoprim, sulfamethoxazole, ciprofloxacin, cefotaxime, ceftazidime, cepoperazone/sulbactam and imipenem, and M-H agar (Oxoid; Thermo Fisher Scientific, Inc., Waltham, MA, USA); 100 bp DNA Ladder marker and D15000+2000 DNA marker (Tiangen Biotech Co., Ltd., Beijing, China); Takara Taq kit, DL2000TM DNA marker and restriction enzyme (Takara Biotechnology Co., Ltd., Dalian, China), agarose (Invitrogen; Thermo Fisher Scientific, Inc.); bacterial identification instrument (ATB Expression; BioMérieux, Marcy l’Etoile, France); DNA amplifier (Biometra GmbH, Göttingen, Germany); electrophoresis apparatus (10C type; Beijing Liuyi Biotechnology Co., Ltd., Beijing, China); biosafety cabinets (1200IIA2; Shanghai Lishen Biotechnology Co., Ltd.). Casein hydrolysate acid (M-H) agar (Shanghai Lishen Biological Technology Co., Ltd.).

**Strain identification and antimicrobial susceptibility assessment.** The preserved strains were removed from the -86°C ultra-low temperature freezer, thawed at room temperature and re-identified. Subsequently, the strains underwent an agglutination test with *Shigella* diagnostic serum for the serotype identification. In total, 3-4 bacterial colonies of *S. flexneri* were detected following culture of the stools for 16-18 h at 35°C, followed by adjustment of the turbidity instrument to 0.5 McF with normal saline. Sterile cotton swabs were dipped into the bacteria solution and excess liquid was squeezed out against the tube wall, followed by application of the bacteria onto the M-H plate. The bacteria were applied onto the M-H plate three times with a 60° rotation; the bacteria were also applied along the edge for entire 360° rotation. Following 5 min, the antimicrobial susceptibility disks were placed on the colonies and cultured for 16-18 h at 35°C. Following these procedures, the inhibition zone diameter was detected with vernier calipers and the data were input into WHONET 5.4 software (http://www.whonet.org/). According to the 2010 version of Performance Standards for Antimicrobial Susceptibility Testing of the National Committee for Clinical Standards Laboratory Institute) (5), the quality control strains was *Escherichia coli* (cat. no. 25922).

**Assessment and sequencing for resistance gene cassettes of integrons and variable regions.** The integron and plasmid DNA templates were prepared using a boiling method for 5 min, and they were preserved in a -20°C refrigerator. The primers and primer sequences were synthesized in the accordance with relative reports (6-9), as in Table I. The class 1, 2 and 3 integrons, integrase primers and variable region primers were synthesized by Takara Biotechnology Co., Ltd. The reaction system was as follows: 10X buffer containing Mg²⁺ (5 µl), dNTP (4 µl; 2.5 mmol/l), downstream and upstream primers (2.5 µl; 10 µmol/l of each), Taq eZzyme (0.25 µl; 5 U/µl) and DNA templates (5 µl). Deionized water was added to the reaction system to 50 µl. The amplification parameters for integrase were as follows: Pre-denaturation at 94°C for 5 min, followed by 94°C for 30 sec, annealing for 30 sec (annealing temperature shown in Table I) and 72°C for 40 sec, for 35 cycles, with final extension at 72°C for 5 min. The amplification parameters for variable regions were as follows: Pre-denaturation at 94°C for 5 min; touchdown polymerase chain reaction (PCR) for 10 cycles, annealing temperature reduction from 60 to 50°C (temperature decrease by 1.4°C each cycle), followed by 94°C for 30 sec, 52°C for 30 sec and 72°C for 180 sec, for 25 cycles with final extension at 72°C for 8 min. For product analysis, the amplification product (10 µl) was used for 1% agarose electrophoresis under 120 V for 20 min. Following EB staining, the products were observed under the UV absorption spectra analyzer. Images were captured and the results recorded. The amplification products were sent to Takara Biotechnology Co., Ltd. for gene sequencing and the sequences were compared with NCBI/BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**BamHI restriction enzyme digestion.** Enzyme digestion was performed in the variable regions of the atypial class 1,2 integrons with the restriction enzyme HindIII and HinfI. The specific steps were as follows: 10 µl enzyme buffer, 40 µl PCR products and 5 µl restriction enzyme were added to deionized water to 100 µl. The solution was placed at 37°C for the isothermal reaction for ~1 h. Subsequently, 10X loading buffer was added to terminate the reaction. The 10-µl samples were used for 2.5% agarose electrophoresis under 120 V for 20 min. Following EB staining, the products were observed under the UV absorption spectra analyzer. Images were captured and the results recorded.

**Analysis of acrAB-tolC and its associated regulatory genes.** The multi-drug resistant *Shigella* strains (resistant to five or more antibiotics) and sensitive strains were used for assessment. The PCR amplification and sequencing methods were applied. The specific steps were in accordance with the above methods. According to previous reports (10,11), the experimental primers were synthesized by Takara Biotechnology Co., Ltd. (Table II). The sequences were compared with NCBI/BLAST to investigate the numbers of mutations and mutational sites.
Results

Results of drug sensitivity tests. A total of 91.1% of the 158 strains were multi-resistant to ampicillin, chloramphenicol, tetracycline and streptomycin, and 69.6% of the strains were multi-resistant to sulfamethoxazole/trimethoprim. The resistance rate to ceftazidime, ciprofloxacin and levofloxacin was <32.9%. All (100%) of the strains were sensitive to cefoxitin, cefoperazone/sulbactam and imipenem. The frequent resistant pattern (ampicillin-tetracycline-chloramphenicol-streptomycin) accounted for 91.1% of strains (144/158), and 77.2% (122/158) of the strains were resistant to more than five antibiotics. Multi-drug resistance was a serious problem. Only 14 strains were sensitive strains, which were not drug-resistant (Table III).

Integration assessment results. Among the 158 S. flexneri strains, 144 strains were intI1-positive and 136 strains were intI2-positive. The positive rates were 91.1% (144/158) and 86.1% (136/158), respectively (Figs. 1 and 2). All strains were intI3-positive. In total, 10 strains were single intI1-positive, accounting for 6.3% (10/158), and 134 strains were intII-intI2 positive, accounting for 84.8% (134/158). Among the intII-positive strains, the 3’-conserved segment of 16.5% of the strains (26/158) were positive. They were typical class 1 integrons [intI1, (conservative primer of class 1 integrons) in and qacEΔ1-sul1-positive; Fig. 3]. The 3’-conserved segment of 84.8% (134/158) of the strains were negative. They were atypical class 1 integrons (intI1 and intI1-IS1-positive; Fig. 4). In addition, 10.1% (16/158) strains (S. flexneri 2A) possessed typical and atypical class 1 integron class 1 integrons. The integron assessment results are shown in Table III.

Assessment and sequencing for resistance gene cassettes of integrons and variable regions. Among the 26 strains...
with typical class 1 integrons, PCR amplification of the variable regions in 22 strains yielded two types of fragment. The sizes were ~1.6 kb and 0.7 kb, respectively (Fig. 3). Confirmed by the gene sequencing, four *S. flexneri* 4a strains carried the *dfrA17-aadA5* (1,624 bp) drug-resistant gene cassette, which was the dihydrofolate reductase gene, and aminoglycoside acyltransferase gene. A total of 18 *S. flexneri* 2a strains carried the *dfrV* (729 bp) drug-resistant gene cassette, which was the dihydrofolate reductase gene and aminoglycoside acyltransferase gene. The PCR amplification

### Table III. Results of class 1 and class 2 integron detection in 79 strains of *Shigella flexneri*.

| Serotype       | Typical class 1 integron (intI1, in, qacEΔ1-sul1-positive) | Atypical class 1 integron (intI1, intI1-IS1-positive) | Class 2 integron (intI2, intI2ca-positive) |
|----------------|-------------------------------------------------------------|-------------------------------------------------------|----------------------------------------|
| *S. flexneri* 1a | 0                                                          | 16                                                   | 14                                     |
| *S. flexneri* 2a | 20                                                         | 70                                                   | 74                                     |
| *S. flexneri* 2b | 2                                                          | 10                                                   | 10                                     |
| *S. flexneri* 4a | 4                                                          | 34                                                   | 34                                     |
| *S. flexneri* x variant | 0                                                         | 4                                                    | 4                                      |
| Total          | 26                                                         | 134                                                  | 136                                    |

---

**Figure 1.** Region of the class 1 integrase polymerase chain reaction electropherogram. M, DNA marker; 1-6 are the specimen numbers.

**Figure 2.** Region of the class 2 integrase polymerase chain reaction electropherogram. M, DNA marker; N, Negative control; 1-6 are the specimen numbers.
of four stains yielded nothing suggest an empty cassette. The assessment of antibiotic susceptibility showed that the drug resistance of pathogens to ampicillin and other drugs was severe, suggesting that the drug resistance was associated with the insertion of a 3' conservative region into the C-terminal conservative region (9). The variable regions of S. flexneri strains were amplified with the \textit{intI1ca-IS1ca} primer. A total of 134 strains yielded 2.4 kb fragments, as shown in Fig. 4. The products had the same enzymatic maps following \textit{Hind}III enzyme digestion. Four strains were randomly selected for sequencing. The carried drug resistant gene cassette was \textit{bla0XA-30-aadA1} (2,453 bp), which was the β-lactamase and aminoglycoside adenosine acyltransferase gene. A total of 134 strains with atypical class 1 integron were resistant to ampicillin, streptomycin, tetracycline and chloromycetin. Only 10 of the 26 strains without atypical class 1 integron were resistant to more than four antibiotics. There was a significant difference between two groups (χ²=35.96, P<0.01). The detailed results of the resistance gene in class 1 integron variable regions are shown in Table III.

Resistance gene cassette assessment and sequencing in variable regions of integrons. The variable region of integrons in the 136 strains was amplified by integron PCR. Following electrophoresis, all the strains yielded 2.2 kb fragments (Fig. 5). Following \textit{hin}f1 digestion, the amplification products manifested the same characteristic bands in electrophoresis. Verified by gene sequencing, the drug-resistant gene carried by bacteria was the \textit{dfrA1-sat1-aadA1} gene (2,224 bp), which was responsible for the drug resistance to trimethoprim, streptomycin and aminoglycoside. The results...
of the resistance gene in class 2 integron variable regions are shown in Table IV.

Analysis of acrAB-tolC and its associated regulatory genes in multi-drug resistant Shigella. There were 12 strains with gene deletions among the 122 multi-drug resistant strains and two strains with gene deletions in 17 sensitive strains. Therefore 110 multi-drug resistant strains and 15 sensitive strains yielded 131, 510, 604, 1,100 and 800 bp sequences (Fig. 6-D). Verified by gene sequencing, these fragments were the PCR amplification products of acrA, acrB, marOR, soxS and acrB. There were 110 cases in drug resistant group and four cases in the sensitive group. The gene analysis showed a low mutation rate in the acrA, acrB, acrR and soxS genes. In the multi-drug resistant strains, a base CATT deletion in the 36, 37, 38 and 39 sites of marOR was detected. However, no marOR mutation was found in sensitive strains.

Discussion

Bacillary dysentery caused by Shigella is a serious intestinal infectious disease and the incidence ranks third among the national statutory B infectious diseases. In addition to transient immunity following infection, the absence of cross-immunoreactivity between subtypes and problems in the immune system of children, the increased drug-resistance of Shigella poses a serious threat to childrens' health. A previous study showed that Shigella had the AcrAB-TolC active efflux pump, which caused multi-drug resistance to organic solvents, dyes, detergents and various antibiotics drugs (12). In another study (13), the high expression level of the active efflux pump can enhance the resistance of bacteria to organic solvents. It also been reported that the resistance of Escherichia coli to organic solvents was affected by certain inherited traits, including the imp/ostA gene, which coded a protein with a molecular mass of 87,000. The protein was attached to the outer membranes. The low expression of the gene increased the sensitivity to N-hexane (14). Certain cell elements that do not belong to the antivie efflux pump, for example the derivatives of the O-sidechain of lipopolysaccharides or the isomers of fatty acid membranes, may have an effect on the resistance of Pseudomonas to organic solvents (15).

The results of the present study showed that 91.1% S. flexneri strains were multi-drug resistant. According to the shigella treatment recommendations of the American Clinical and Laboratory Standards Institute, ampicillin, sulfamethoxazole/trimethoprim were not suitable for the empirical clinical medication. Although the drug resistance rate of ciprofloxacin and levofloxacin was 32.9%, the fluoroquinolones were not suitable for pediatric patients due to the potential damage to cartilage in infants and children. The drug-resistance mechanism was associated with multiple-site mutations of gene gyrA and/or parC. The drug-resistance rate to cefotaxime and cefotaxime was <30.4%, which meant that these drugs were suitable for use in the treatment of bacillary dysentery in children. It is noteworthy that the drug resistance was significantly higher than that reported previously In China and elsewhere (16,17), sugesting that, in case of treatment failure with third generation cephalosporins or in case of pediatric dental patents, cefoxitin, cefoperazone/sulbactam and imipenem may be applied in the treatment.

The integron-mediated multidrug-resistance has been verified previously. Class 1 integron was detected in 91.1% of 158 S. flexneri strains. Class 2 integron was detected in 86.1% of 158 S. flexneri strains. The propotions were significantly higher, compared with those reported in South Korea and Iran (18,19). The isolating regions and epidemic strains may accout for this. The typical class 1 integron gene was present in 15.2% of the isolates screened. The drug resistance gene cassette was frv and dfrA17-aadA5. The atypical class 1 integron accounted for 86.1% strains. The drug resistance gene cassette was blaOXA30-aadA1. The typical and atypical class 1 integrons were present in 10.1% of isolates screened, similar with the relative reports (20,21). The typical class 1 integron was present in 11.4% of the S. flexneri isolates screened. This result has not been reported previously, to the best of our knowledge. In individual studies, the dfrv gene was present in Escherichia coli, Salmonella bacteria and Gram-negative bacilli in waste water (20-22). Whether the resistance genes were transfered from these bacteria requires further investigation.
A total of 134 strains with atypical class 1 integron were resistant to ampicillin, streptomycin, tetracycline and chloramphenicol. Only 10 of the 26 strains without the atypical class 1 integron were resistant to more than four antibiotics. There was a significant difference between them ($\chi^2=35.96$, P<0.01). This difference is associated with a situation that the $blaOXA-30-aadA1$ gene cassette in the atypical class 1 integron variable region was adjacent to the chloramphenicol- and tetracycline-resistant determinants (23). Those strains with no atypical class 1 integrons, which were resistant to the
four antibiotics may have other drug resistance mechanisms. There were 10.1% strains (S. flexneri 2A) possessing typical and atypical class 1 integron class 1 integrons (24), which deteriorate the drug resistance of S. flexneri. The typical class 1 integron was found in conjugative plasmids and can mediate the horizontal gene transfer. In the present study, 86.1% S. flexneri isolates carried the class 2 integron and the gene cassette was dfrA1-sat1-aadA1, which was consistent with other reports (25,26). The gene cassettes reported currently comprise dfrA1-sat1, dfrA1-sat2-aadA1 and sat2-aadA1 (27-29). As the class 2 integron integrase is inadequate for integrating novel resistance genes from externally or to resect the resistance genes from its own variable region, the drug resistance gene cassettes of class 2 integron variable regions are relatively conservative.

The drug resistance rate to sulfamethoxazole/trimethoprim was 69.6%. The strains resistant to sulfamethoxazole/trimethoprim were compared with the sul gene carried in the atypical class 1 integron, the dfrA1 gene carried in the class 2 integron, and the two together, suggesting that the two genes were not associated with the drug resistance. The two genes may be nonfunctional structural genes. The mechanism underlyng the drug resistance to sulfamethoxazole/trimethoprim requires further investigation.

The single-stranded conformation polymorphism suggested a low mutation rate of acrR, acrB and tolC in drug-resistant Shigella. The expression of AcrA and AcrB are regulated by several regulators. Of all the regulators the acrR suppressors and marOR operon are important. The results demonstrated a low mutation rate of marOR in drug-resistant Shigella, suggesting that the mutation of the regulatory gene contributed more to the drug resistance of Shigella than the mutation of a pump gene. Further investigations aim to examine the association between the mutation of regulatory gene marOR and the expression of acrA, acrB and tolC, which may provide a theoretical basis for the drug-resistant mechanism mediated by the active efflux pump of Shigella.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

QPM was responsible for specimen collection, bacterial identification, and experimental procedures. MXY was responsible for experimental operations and data collection. LS and JWL were responsible for data collection and statistical analysis. GYY was responsible for experimental design and data review.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jinan Infectious Disease Hospital Affiliated To Shandong University (Shandong, China).

Consent for publication

Written informed consent was obtained from all patients or patient guardians for the present study.

Competing interests

The authors declare they have no competing interests.

References

1. Partridge SR: Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol Rev 35: 820-855, 2011.

2. Poole K: Efflux-mediated multiresistance in Gram-negative bacteria. Clin Microbiol Infect 10: 12-26, 2004.

3. Yang HY, Duan GC and Xi YL: Distribution and expression of efflux pump acrAB in clinical isolates of Shigella. Chin J Public Health 20: 685-687, 2005.

4. Lv RL, Duan G and Ca and Song CH: Analysis on acrR, marOR multi-drug regulated gene mutations in clinical isolates of Shigella. Chin J Public Health 24: 865-965, 2008.

5. Cockerill FR, Cockerill F, Adler J, Dudley MN, Patel JB, Eliopoulos GM and Bradford PA: Performance standards for antimicrobial susceptibility testing: Twenty-first informational supplement. Clin Lab Stand Inst 31: 41-42, 2011.

6. White PA, McLver CJ and Rawlinson WD: Integrons and gene cassettes in the enterobacteriaeae. Antimicrob Agents Chemother 45: 2658-2661, 2001.

7. Ploy MC, Denis F, Courvalin P and Lambert T: Molecular characterization of integrons in Acinetobacter baumannii: Description of a hybrid class 2 integron. Antimicrob Agents Chemother 44: 2684-2688, 2000.

8. Dalsgaard A, Forslund A, Serichantalergs O and Sandvang D: Distribution and content of class 1 integrons in different Vibrio cholerae O-serotype strains isolated in Thailand. Antimicrob Agents Chemother 44: 1315-1321, 2000.

9. Pan JC, Ye R, Meng DM, Zhang W, Wang HQ and Liu KZ: Molecular characteristics of class 1 and class 2 integrons and their relationships to antibiotic resistance in clinical isolates of Shigella sonnei and Shigella flexneri. J Antimicrob Agents 58: 288-296, 2006.

10. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, et al: Relation of acrAB-tolC efflux pump and marOR regulatory gene mutation with antimicrobial resistance in Shigella, spp. J Jilin Univ 36: 45-48, 2010.

11. Rahman M, Mauff G, Levy J, Couturier M, Pulvorer G, Glasdorf N and Butzler JP: Detection of 4-quinolone resistance mutation in gyrA gene of Shigella dysenteriae type 1 by PCR. Antimicrob Agents Chemother 38: 2488-2491, 1994.

12. Abe S, Okutsu T, Nakajima H, Kakuda N, Ohtsu I and Aono R: n-Hexane sensitivity of Escherichia coli due to low expression of impA-tsta encoding an 87 kDa minor protein associated with the outer membrane. Microbiology 149: 1265-1273, 2012.

13. Sikkema J, de Bont JA and Poolman B: Mechanism of membrane toxicity of hydrocarbons. Microbiol Rev 59: 201-222, 1995.

14. Wang XN, Wei DJ, Zhang XL and Li WS: Study on extended-spectrum 6-lactamases and their genotypes of Shigella isolated from Tianjin. Chin J Infect Dis 36: 71-75, 2012.

15. Varghese SR and Aggarwal A: Extended spectrum beta-lactamase production in Shigella isolates-a matter of concern. Indian J Med Microbiol 29: 76-78, 2011.

16. Hong S, Choi YH, Choo Y, Choi Y, Cho SY, Kim DW, Lee BK and Park MS: Genetic characterization of atypical Shigella flexneri isolated in Korea. J Microbiol Biotechnol 20: 1457-1462, 2010.
17. Eftekhari N, Bakhshi B, Pourshafie MR, Zarbakhsh B, Rahbar M, Hajia M and Ghazvini K: Genetic diversity of Shigella spp. and their integron content. Foodborne Pathog Dis 10: 237-242, 2013.
18. Schaumburg F, Alabi AS, Kaba H, Lell B, Becker K, Grobusch MP, Kremsner PG and Mellmann A: Molecular characterization of Shigella spp. from patients in Gabon 2011-2013. Trans R Soc Trop Med Hyg 109: 275-279, 2015.
19. Gassama Sow A, Aidara-Kane A, Barraud O, Gatet M, Denis F and Ploy MC: High prevalence of trimethoprim-resistance cassettes in class 1 and 2 integrons in Senegalese Shigella spp isolates. J Infect Dev Ctries 4: 207-212, 2010.
20. Kim TE, Jeong YW, Cho SH, Kim SJ and Kwon HJ: Chronological study of antibiotic resistances and their relevant genes in Korean avian pathogenic Escherichia coli isolates. J Clin Microbiol 45: 3309-3315, 2007.
21. Nógrády N, Gado I, Tóth A and Pászti J: Antibiotic resistance and class 1 integron patterns of non-typhoidal human Salmonella serotypes isolated in Hungary in 2002 and 2003. Int J Antimicrob Agents 26: 126-132, 2005.
22. Rosser SJ and Young HK: Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J Antimicrob Chemother 44: 11-18, 1999.
23. Luck SN, Turner SA, Rajakumar K, Sakellaris H and Adler B: Ferric dicitrate transport system (Fec) of Shigella flexneri 2a YSH6000 is encoded on a novel pathogenicity island carrying multiple antibiotic resistance genes. Infect Immun 69: 6012-6021, 2001.
24. Zhu JY, Duan GC, Yang HY, Fan QT and Xi YL: Atypical class 1 integron coexists with class 1 and class 2 integrons in multi-drug resistant Shigella flexneri isolates from China. Curr Microbiol 62: 802-806, 2011.
25. Huang YL, Xu YL, Zhuang L, Qian HM, Gu L, Gu B, Liu GY and Pan SY: Molecular epidemiology and integron analysis of Shigella flexneri isolates from Jiangsu province in 2011. Chi J Clin Infect Dis 7: 34-38, 2011.
26. Wang J, Liang F, Wu XM and Qi W: Transfer patterns of integron-associated and antibiotic resistance genes in S. flexneri during different time intervals in Tianjin, China. Indian J Med Microbiol 32: 256-260, 2014.
27. Xia R, Ren Y, Guo X and Xu H: Molecular diversity of class 2 integrons in antibiotic-resistant gram-negative bacteria found in wastewater environments in China. Ecotoxicology 22: 402-414, 2013.
28. Chang CY, Lu PL, Lin CC, Lee TM, Tsai MY and Chang LL: Integron types, gene cassettes, antimicrobial resistance genes and plasmids of Shigella sonnei isolates from outbreaks and sporadic cases in Taiwan. J Med Microbiol 60: 197-204, 2011.
29. Ud-Din AI, Wahid SU, Latif HA, Shahnaij M, Akter M, Azmi IJ, Hasan TN, Ahmed D, Hossain MA, Faruque AS, et al: Changing trends in the prevalence of Shigella species: Emergence of multi-drug resistant Shigella sonnei biotype g in Bangladesh. PLoS One 8: e82601, 2013.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.