PCR-Based Identification of Integrons Types and Extended-Spectrum B-Lactamase Genes in *Salmonella* Species Collected from Pediatric Diarrheal Samples

Pediatrik İshal Örneklerinden Toplanan *Salmonella* Türlerinde İntegran Tiplerinin ve Genişletilmiş Spektrumlu B-Laktamaz Genlerinin PCR Tabanlı Tanımlanması

Hamid Eshaghi [1], Saman Alhooei [2,3], Arash Abbasi [4], Mahmoud Khodabandeh [1], Fatemeh Hejazi Amiri [5], Abazar Pournajaf [6] Masoumeh Kiani [7], Mehrdad Gholami [8], Peyman Hendizade [5], Mohsen Mohammadi [9]

[1] Department of Infectious Diseases, Pediatric's Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Internal Medicine, School of Medicine, Babol University of Medical Sciences, Babol, Iran.
[3] Clinical Research Development Unite of Rouhani Hospital, Babol University of Medical Sciences, Babol, Iran.
[4] Pediatric Chronic Kidney Disease Research Center, The Children's hospital Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[5] Department of Microbiology, School of Medicine, Babol University of Medical Sciences, Babol, Iran
[6] Infectious Diseases and Tropical Medicine Research Center, Babol University of Medical Sciences, Babol, Iran
[7] Department of microbiology, faculty of medicine, Shahid Sadoughi University of medical sciences, Yazd, Iran.
[8] Department of Microbiology and virology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[9] Noncommunicable Pediatric Diseases Research Center, Health Research institute, Babol University of Medical Sciences, Babol, Iran

ABSTRACT

Objective: *Salmonella* is a significant pathogen between food-borne diseases. *Salmonella* spp. strains that produce extended-spectrum β-Lactamases (ESBL) have become a medical problem for both antimicrobial therapy and infection control programs. The aim of this project was detection of ESBL genes and class I, II and III Integrons in the *Salmonella* isolates.
Methods: 405 non-duplicative stool samples were obtained. Antibacterial susceptibility was defined by the disk diffusion and also double disk synergy test (DDST) was used for confirming of ESBL phenotype. The multiplex-PCRs were directed for recognition of ESBLs (*TEM*, *CTX-M* and *SHV*) and *int* (I, II, III) genes.
Results: Out of 405 samples, 54 (13.4%) *Salmonella* were obtained. The highest resistance rate was related to the NA (51.8%), followed by SXT (50%), CTX (46.3%), and AMP (33.3%). DDST was conducted for all isolates and 7 (12.9%) Salmonella spp were ESBL positive. Molecular analysis showed that 5 (9.3 %) of isolates were carried *blaTEM-1* which belonged to the *S. infantis* and *S. typhimurium*. Three (5.5%) non-typeable isolates and 2 *S. typhimurium* were positive for the CTX-M gene. The prevalence of different classes of integrons showed that 23 (42.5%) isolates carried the integrase (*int*) gene.
Conclusion: This research demonstrates the predominant existence in the *Salmonella* of the TEM and CTX-M genes. So, class I integron were more prevalent than class II and III in *Salmonella* isolates. They are capable of transferring to bacteria of this genus and also the other genus of intestinal ones.

Keywords: *Salmonella*, extended spectrum β-lactamases, integron.

Received: 02.27.2020 **Accepted:** 10.21.2020

ÖZET

Amaç: *Salmonella*, gıda kaynaklı hastalıklar arasında önemli bir patojendir. *Salmonella spp*. genişletilmiş spektrumlu β-laktamazlar (ESBL) üreten suşlar, hem antimikrobiyal tedavi hem de enfeksiyon kontrol programları için tıbbi bir sorun haline gelmiştir. Bu projenin amacı, *Salmonella* izolatlarında GSBL genlerinin ve sınıf I, II ve III İntegronların saptanmasıdır.
Yöntemler: Toplam 405 duplikatif olmayan dışkı örneği elde edildi. Antibakteriyel duyarlılık disk difüzyonu ile tanımlandı ve ayrıca GSBL fenotipinin doğrulanması için çift disk sinerji testi (DDST) kullanıldı. Multipleks-PCR'ler, ESBL'lerin (TEM, CTX-M ve SHV) ve int (I, II, III) genlerinin tanınmasına yöneliktir.
Bulgular: Toplam 405 örnekten 54'ü (%13.4) Salmonella elde edildi. En yüksek direnç oranı NA (%51,8) ile ilgiliydi, bunu SXT (%50), CTX (%46,3) ve AMP (%33,3) izledi. Tüm izolatlara DDST uygulandı ve 7 (%12.9) *Salmonella spp* GSBL pozitifti. Moleküler analizler, 5 (%9.3) izolatın S. infantis ve S. typhimurium'a ait blaTEM-1 taşıdığını gösterdi. Üç (%5.5) tiplenemeyen izolat ve 2 S. typhimurium, CTX-M geni için pozitifti. Farklı integron sınıflarının prevalansı 23 (%42.5) izolatın integraz (int) genini taşıdığını göstermiştir.
Sonuç: Bu araştırma, TEM ve CTX-M genlerinin *Salmonella*'da baskın olduğunu göstermektedir. Dolayısıyla, Salmonella izolatlarında sınıf I integron, sınıf II ve III'e göre daha yaygındı. Bu cinsin bakterilerine ve ayrıca bağırsakların diğer cinsine aktarabilirler.

Anahtar Sözcükler: S*almonella*, genişletilmiş spektrumlu β-laktamazlar, integron

Geliş Tarihi: 27.02.2020 **Kabul Tarihi:** 21.10.2020

ORCID IDs: H.E.0000-0003-1420-0903, S.A.0000-0001-6721-9478, A.A.0000-0002-0008-2937, M.K.0000-0002-1567-5284, F.H.A.0000-0001-5427-9594, A.P.0000-0002-6753-5953, M.K.0000-0002-3919-2973, M.G.0000-0003-4251-2488, P.H.0000-0002-1519-8182, M.M.0000-0002-7907-3418

Address for Correspondence / Yazışma Adresi: Dr. M. Mohammadi; Noncommunicable Pediatric Diseases Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran. E-mail: dr.mohamadi61@yahoo.com

doi:http://dx.doi.org/10.12996/gmj.2021.93

INTRODUCTION

Salmonella spp. are recognized as major food-borne pathogen in worldwide and its outbreaks are frequently related to the ingesting of contaminated food (1,2). In both developed and developing countries, human salmonellosis has been a major concern for public health. An estimated 800,000 to 4 million non-typhoidal salmonellosis (NTS) are recorded per year in the United States, and about 500 of them die (3). The most consequence of this infection is acute gastroenteritis and does not require to antibiotic therapy (4). However, chemotherapy is commonly suggested for cases with salmonellosis, mostly those cases at high risk of systemic infection, such as; elderly, pregnant, immunocompromised patients and children less than 1 year of age (3-5). The extensive misuse and misappropriation of antimicrobials in domestic animals and food additives have contributed to the increase of drug resistant pathogens such as *Salmonella* (6). Proper antibiotic therapy for non-typhoidal salmonellosis illnesses may reduce of severity and infection period and may also prevention of fatality and further illness transmission (7). The actual concerns are those strains that have acquired multiple drug resistance (MDR) against three or more classes of therapeutic agents (8). Extended-spectrum cephalosporins (ESCs) are counted as an alternative therapeutic choice for NTS infections that are resistant to treatment. The increased trend of using β-lactam drugs to treat intestinal diseases had developed penicillin and cephalosporin resistance in *Salmonella* spp. in different parts of the world and had been a reason for drug therapeutic failure (DTF) (9,10). Usually, extended-spectrum beta-lactamases (ESBLs) are encoded by a large-sized plasmid moving from inter and intra species of bacteria (11). Resistance to ESCs is primiraliy promoted by generation of class A ESBLs, that capable of hydrolyzing oxyimino cephalosporins but are not active against carbapenems and cephamycins. Temoneira (TEM), cefotaximase (CTX-M) and sulfhydryl variable (SHV) are found in class A ESBLS. SHV type β-lactamases are associated with high ceftazidime resistance, but not with cefazolin and cefotaxime, whereas CTX-M β-lactamases are more efficient against cefotaxime.TEM β-lactamases,by comparison, impart resistance to oxyimino-β-lactams groups, like ceftazidime, cefotaxime, and aztreonam (12,13). Instead of ESBLs, the mobile genetic elements (MGEs), including integrons, can evolve and distribute resistance genes in NTSs. Integrons are conserved segments (3′-CS and 5′-CS) genetic units categorized by their capacity to harbor and integrate gene cassettes by site-specific recombination. Three classes of antibiotic resistance integrons (ARIs) (classes I, II, and III) have been mostly recognized in the MDR phenotypes criteria and are identified according to integrase (*int*) genes (14, 15). The transferable class I (Tn21 and Tn402 derivatives) integrons are the most common type and followed by class II and III, respectively (16). Class I integrons harbor many antimicrobial gene cassettes encoding ESBLs, *dfr*, qacEΔ1, *sul1* and aminoglycoside-modifying enzymes (AMEs) (17, 18). Class II Integron located on the Tn7 and 3′-CS contains five *tns* genes which are corresponding for the elasticity of transposons (TE). Integron of class III situated in a TE, but the 3′-CS is unknown (19). The objective of the this research project was detection of *blaTEM*, *blaCTX-M*, *blaSHV*, and *int* genes (class I, II and III integrons) in the NTS strains isolated from in pediatric patients by multiplex PCR (M-PCR) and their antibiotic resistance profile.

MATERIALS and METHODS

Study design and Ethical approval

This work was financially supported by a grant (no. 724133739) by Babol University of Medical Sciences, Babol, Iran. Written informed consent form was collected from the patient's parents. Identifying information of each sample was kept secret.

Bacterial isolates

In the cross-sectional study, 405 non-duplicative stool samples were collected from the pediatric patients with aged less than 10 years. Samples were cultured on the Mac Conkey agar (Merck, Germany) and incubated at 37 °C for 24 h. Then, all suspected grown colonies were recognized as *Salmonella* by conventional biochemical and microbiological tests and confirmed by the API 20E system (Analytab, New York). Serotyping with specific O and H *Salmonella* antisera was performed according to the by slide agglutination method (Denka Seiken, Japan).

Antibiotic susceptibility test (AST)

Antibiotic susceptibility test was performed by disc diffusion (DD) method on the Mueller- Hinton Agar (Merck, Germany) plates according to the Clinical and Laboratory Standards Institute (CLSI M100-S28) guideline for the following antibiotics: amoxicillin/clavulanate (AMC; 20/10 µg), ciprofloxacin (CIP: 5 µg), amikacin (AK: 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), cefotaxime (CTX: 30 µg), ampicillin (AMP: 10 µg), aztreonam (ATM: 30 µg), imipenem (IPM: 10 µg), gentamicin (GM: 10 µg), ceftazidime (CAZ: 30 µg), cefoxitin (FOX: 30 µg), ceftriaxone (CRO: 30 µg), chloramphenicol (CHL: 30 µg), nalidixic acid (NA: 30 µg), tetracycline (TET: 30 µg) and ofloxacin (OFX: 5µg) (Mast, Merseyside, UK). In short, Bacterial suspension prepared to match the turbidity of the 0.5 McFarland turbidity standard and then cultured on Mueller-Hinton agar (Oxoid, UK). Inhibition zone diameters were measured after incubation time and the results were reported as susceptible, intermediate, and resistant. Double disk synergy test (DDST) was used for screening of ESBL strains. The combination disk test based on the clavulanic acid inhibitory effect was also used according to the CLSI guideline (13). *E. coli* ATCC 25922 was used as a reference strain for antimicrobial susceptibility test.

Molecular Analyses

M-PCRs were performed by using the DNA amplification instrument master cycler gradient(Eppendorf Co., Germany) for identification of ESBLs genes (bla_{TEM}, bla_{CTX-M} and bla_{SHV}) and *int* genes (I, II and III). We used ESBLs and integrons primer as mentioned previously (12,20). DNA extraction for all *Salmonella* strains performed by using the boiling lysis method. Concisely, a loopful of bacterial colonies was suspended in the 1000 µl distilled water (DW) and boiled for 15 minutes and centrifuged at 7000×g for 5 minutes at 4°C and then cooling in ice for 15 minutes and centrifugation for 4 min at 8000×g. The DNA quality was measured by using the Nanodrop spectrophotometer (ND-1000; Thermo Scientific; Wilmington, DE, USA). M-PCR was done for amplification of ESBLs genes (bla_{TEM}, bla_{CTX-M} and bla_{SHV}) in a volume of 1.3 µl of extracted genomic DNA was added to a final volume of 25 µl PCR reaction mixture comprising 2.0 µl of 10× PCR buffer, 1.5 µl $MgCl_2$ (50 mM), 0.5 µl dNTPs (10 mM), 1.0 µl of each primer, 0.5 µl of Taq DNA polymerase (5 U/µl) (Amplicon Co., Denmark) and 13.2µl DW. The reaction mixture was done with the following procedure: denaturation at 94°C for 60 seconds, 33 cycles with denaturation at 94°C for 45 seconds, annealing at 59°C for 30 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 5 minutes. So, amplification of *int* genes (I, II and III), the reaction mixture was completed in a thermal gradient cycler (Eppendorf Co., Germany) with the following PCR protocol: The cycling conditions were one cycle of 5 minutes at 95 °C; 30 cycles of 1 minutes at 95°C, 1 minutes at 65°C, and 1 minutes at 72°C; and one cycle of 10 minutes at 72°C. Briefly, the whole 25 µl volume of MPCR reaction mixture for all genes (*intI*, *intII* and *intIII*) contained of 1× PCR buffer, 3.0 mM of $MgCl_2$, 2.0 mM of each dNTPs, 3U of Taq polymerase, 10 pmol of each primer, 2.5 µl of DNA template and distilled water to reach the reaction volume (12,20). The amplification products were separated by agarose gel electrophoresis by using 1.5% agarose gel stained with ethidium bromide (EtBr) and imagined with UV Gel documentation system (UVP Gel Seq Software, England).

Statistical analysis

The collected data were statistically analyzed using SPSS program (Version 16.0). Data were subjected to descriptive statistics and expressed in percentages.

RESULTS

Out of 405 stool samples, 54 (13.4%) *Salmonella* were obtained. The main serotypes was *S.* Enteritidis (n; 24, 44.5%) followed by *S*. Typhimurium (n; 13, 24%), *S*. Infantis (n; 6, 11%), *S*. Bardo (n; 3, 5.5%), *S*. Heidelberg (n; 2, 4%) and (n; 6, 11%) NTS. In general 193 (47.6 %) of cases were male and 212 (52.4 %) were female. Our results indicated that the highest resistance rate were related to the NA (51.8%), followed by SXT (50%), CTX (46.3%), and AMP (33.3%). All isolates were susceptible to CIP, IPM, OFX and AK (Table 1). DDST results showed that, 7 (12.9%) of *Salmonella* isolates were producing ESBL. Molecular analysis of ESBLs genes showed results, 5 (9.3 %) of isolates were carried bla_{TEM-1} which belonged to the *S*. Infantis and *S*. Typhimurium. Three (5.5%) non-typeable isolates and 2 (3.7%) *S*. Typhimurium strains were positive for the *CTX-M* gene.

Nevertheless, it is notable that the *blaSHV* gene was not identified in any of the *Salmonella* spp. The prevalence of different classes of integrons showed that 23 (42.5%) isolates carried the integrase (*int*) gene. The highest frequency of intI was found in *S*. Enteritidis (n; 13, 56.5%) followed by *S*. Infantis (n; 6, 26%) and *S*. Typhimurium (n; 4, 17.5%). So, 6 strains including, 4 (66.7%) *S*. Enteritidis and 2 (33.3) *S.* Infantis carried class II integron. No class III integron-positive isolates were detected by M-PCR analysis. In the present study, *S*. Bardo and *S*. Heidelberg were negative for *int* (*I, II* and *III*) and ECBLs genes.

Table 1. Antimicrobial susceptibility pattern in the tested strains

Antimicrobial agents	Susceptible	Intermediate	Resistant
CTX	29 (53.7)	0 (0.0)	25 (46.3)
CRO	49 (90.7)	0 (0.0)	9 (16.6)
CAZ	51 (94.4)	0 (0.0)	3 (5.5)
NA	20 (37)	6 (11.1)	28 (51.8)
ATM	47 (87)	0 (0.0)	7 (12.9)
CIP	54 (100)	0 (0.0)	0 (0.0)
IPM	54 (100)	0 (0.0)	0 (0.0)
CHL	38 (70.4)	5 (9.3)	16 (29.6)
SXT	20 (37)	7 (12.9)	27 (50)
OFX	54 (100)	0 (0.0)	0 (0.0)
AMP	28 (51.8)	8 (14.8)	18 (33.3)
GM	48 (88.8)	3 (5.5)	3 (5.5)
FOX	50 (92.6)	2 (3.7)	2 (3.7)
AK	54 (100)	0 (0.0)	0 (0.0)
TET	45 (83.3)	5 (9.3)	4 (7.4)
AMC	47 (87)	0 (0.0)	7(12.9)

CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; NA, nalidixic acid; ATM, aztreonam; CIP, ciprofloxacin; IPM, imipenem; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; OFX, ofloxacin; AMP, ampicillin; GM, gentamicin; FOX, cefoxitin; AK, amikacin; TET, tetracycline; AMC; amoxicillin-clavulanic acid.

DISCUSSION

In the present study, we performed antibiotic susceptibility and detection ESBLs and integron genes among *Salmonella* spp., isolated from pediatric feces samples. In total, 54 *Salmonella* spp., strains were analyzed throughout the project. Our prevalence of *Salmonella* spp., in stool samples was obtained 13.3%. These results are in agreement with the previous study (21). The present study demonstrated that typhoidal salmonellosis is more predominant than non-typhoidal salmonellosis and this result is like the reports by Sood et al. (22) on "Salmonellosis in developing countries". However, in Nigeria, NTS is more common than typhoidal salmonellosis. (21) Our results indicated the high frequency of *S*. Enteritidis strains in pediatric stool samples (n; 24, 44.5%), these findings was agreement with previous reports (21, 23-25). The higher number of *Salmonella* documented versus age-group 0 to 5 years and 6 to 15 years showed that salmonellosis manifested more in children than adults (21, 23-25). All *Salmonella* spp. collected was tested for antimicrobial susceptibility with 16 antibiotics in the present study. Of the total isolates, 51.8% were found to be resistant to NA, 50% resistant to SXT, 46.3% resistant to CTX, 33.3% resistant to AMP, 29.6% resistant to CHL, 12.9% resistant to both ATM and AMC, 9.3% resistant to CRO, 16.6% resistant to CAZ, 7.4% resistant to TET and 3.7% resistant to FOX. In a study conducted by Abdullahi et al (21), 94.2%, 72.8%, 31.8%, 22.2% and 4.9% of isolates were resistance to AMP, CHL, SXT, CRO and NA, respectively. The resistance rate in Salimian Rizi et al (26) study reported were 63.6 % to SXT, 47.3 % to NA, 6.4 % to CRO and CAZ, and 2.7 % were resistant to CTX. Our findings indicate that the high antibiotic resistance was belongs to NA, these finding was concordance with Shrestha et al and Garg et al studies (27, 28). Ranjbar et al (20) reported that most prevalent resistances were associated with doxycycline (64.7%), NA (61.2%), TET (51.8%), and streptomycin (42.8%). Additionally, our 54 strains of *Salmonella* spp exhibited susceptible to the all antimicrobial agents used, such as CIP, OFX, IPM, TN and AK. In similarity to our study, 100 % susceptibility of *Salmonella spp* to the CRO, IPM and OFX were observed in several studies (20, 29-31) . In our study, 29 strains out of 54 (53.7 %) *Salmonella* spp showed either a class 1 integron or class 1 and 2 integron. While, in all strains tested by M-PCR ,integron class 3 was not detected. This distribution of our integron positive isolates approximately is consistent with previous reports that *Salmonella* carried high prevalence of integron class 1, lower class 2 and no class 3 (20, 32-35). This incidence was higher in comparison with a study conducted by Cabrera et al. (36), who exhibited that 25% of *S*. Enterica confined class 1 integrons in Spain. Other research in the United Kingdom discovered that the occurrence of class 1 integrons was 20.4% in *Salmonella* spp. (14), 13% in Hong Kong (37), and 13% in Vietnam (38). In addition to integron genes, ESBLs gene was identified in 10 (18.5%) our isolates of *Salmonella* spp. and also the main enzymes found in them were CTX-M and TEM. While SHV was not developed by any clinical isolates. This prevalence is much higher than any frequency rates of *Salmonella* spp. reported, as recorded in Taiwan by 1.5 percent (39, 40), 1.9% in the USA (41), 2.4% in Latin America (42), 0.8% in Europe and 3.4% in the Western Pacific region (42). The reason behind this high incidence of ESBL-producing *Salmonella* in our study could be attributed to the different rates of antibiotic using in society (43). In 2005, Moubareck and colleagues (44) described for the first time an ESBL-producing *S*. Typhimurium of CTX-M-15-type in the Lebanon. None of our isolates included *blaSHV*, these findings are agreeing with the previous study (43). In Elumalai et al (12) study, blaTEM-1 was identified in 4.47% (n; 6/134) of *Salmonella* spp, which belonged to the *S. Typhi* serotype. All six TEM-positive strains were negative for the *blaSHV* gene and none of the isolates was found positive about the presence of the *blaCTX-M* gene.

CONCLUSION

As in our *Salmonella* strains, the high frequency of integron-positive isolates has shown that these mobile genetic factors are widespread among various *Salmonella* spp. and associated with reducing susceptibility for first-choice antibiotic therapy.

Conflict of interest

No conflict of interest was declared by the authors.

Acknowledgments

The authors would like to thank the Amirkola Children Hospital (Babol, north of Iran) and Children's Medical Center Hospital (Tehran, Iran) for providing samples and for their collaboration in this research.

REFERENCES

1.McEvoy J, Doherty A, Sheridan J, Blair I, McDowell D. The prevalence of Salmonella spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. J Appl Microbiol 2003;94:693-700.

2.Eshaghi M, Bibalan MH, Pournajaf A, Gholami M, Talebi M. Detection of New Virulence Genes in mecA-positive Staphylococcus aureus Isolated from Clinical Samples: The First Report from Iran. Infect Dis Clin Pract. 2017;25:310-3.

3.Acheson D, Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis 2001;32:263-9.

4.Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis 2010;50:882-9.

5.Flint JA, Van Duynhoven YT, Angulo FJ, DeLong SM, Braun P, Kirk M, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clin Infect Dis 2005;41:698-704.
6.Hasannejad-Bibalan M, Mojtahedi A, Biglari H, Halaji M, Sedigh Ebrahim-Saraie H. Antibacterial Activity of Tedizolid, a Novel Oxazolidinone against Methicillin-Resistant Staphylococcus aureus: A Systematic Review and Meta-Analysis. Microb Drug Resist 2019;25:1330-7.
7.Forshell LP, Wierup M. Salmonella contamination: a significant challenge to the global marketing of animal food products. Rev sci tech Off int Epiz 2006;25:541-54.
8.Greene SK, Stuart AM, Medalla FM, Whichard JM, Hoekstra RM, Chiller TM. Distribution of multidrug-resistant human isolates of MDR-ACSSuT Salmonella Typhimurium and MDR-AmpC Salmonella Newport in the United States, 2003-2005. Foodborne Pathog Dis 2008;5:669-80.
9.Nikaido H, Basina M, Nguyen V, Rosenberg EY. Multidrug Efflux Pump AcrAB of Salmonella typhimuriumExcretes Only Those β-Lactam Antibiotics Containing Lipophilic Side Chains. J Bacteriol 1998;180:4686-92.
10. Li X-Z, Mehrotra M, Ghimire S, Adewoye L. β-Lactam resistance and β-lactamases in bacteria of animal origin. Vet Microbiol 2007;121:197-214.
11. Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar N. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing Salmonella enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int J Infect Dis 2006;10:434-8.
12. Elumalai S, Muthu G, Selvam REM, Ramesh S. Detection of TEM-, SHV-and CTX-M-type β-lactamase production among clinical isolates of Salmonella species. J Med Microbiol 2014;63:962-7.
13.CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018
14. Randall L, Cooles S, Osborn M, Piddock L, Woodward MJ. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the UK. J Antimicrob Chemother 2004;53:208-16.
15. Vo AT, Van Duijkeren E, Fluit AC, Wannet WJ, Verbruggen AJ, Maas HM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among non-typhoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2006;28:172-9.
16. Miko A, Pries K, Schroeter A, Helmuth R. Molecular mechanisms of resistance in multidrug-resistant serovars of Salmonella enterica isolated from foods in Germany. J Antimicrob Chemother 2005;56:1025-33.
17. Szmolka A, Lestár B, Pászti J, Fekete PZ, Nagy B. Conjugative IncF and IncI1 plasmids with tet (A) and class 1 integron conferring multidrug resistance in F18+ porcine enterotoxigenic E. coli. Acta Vet Hung 2015;63:425-43.
18. Liamthong S. Prevalence of Class 1 Integrons and Antibiotic Resistance Patterns in Bacteria of Swine and Chicken in the US and Thailand. 2008.
19. Partridge SR, Brown HJ, Stokes H, Hall RM. Transposons Tn1696 and Tn21and their integrons In4 and In2 have independent origins. Antimicrob Agents Chemother 2001;45:1263-70.
20. Ranjbar R, Giammanco GM, Farshad S, Owlia P, Aleo A, Mammina C. Serotypes, antibiotic resistance, and class 1 integrons in Salmonella isolates from pediatric cases of enteritis in Tehran, Iran. Foodborne Pathog Dis 2011;8:547-53.
21. Abdullahi B, Abdulfatai K, Wartu J, Mzungu I, Muhammad HD, Abdulsalam A. Antibiotics susceptibility patterns and characterization of clinical Salmonella serotypes in Katsina State, Nigeria. Afr J Microbiol Res 2014;8:915-21.
22. Sood S, Kapil A, Das B, Jain Y, Kabra S. Re-emergence of chloramphenicolsensitive Salmonella typhi. The Lancet. 1999;353:1241-2.
23. Oracz G, Feleszko W, Golicka D, Maksymiuk J, Klonowska A, Szajewska H. Rapid diagnosis of acute Salmonella gastrointestinal infection. Clin Infect Dis 2003;36:112-5.
24. Sousa MÂB, Mendes EN, Penna FJ, Péret-Filho LA, Magalhães PP. Acute diarrhea associated with Salmonella enterica in Belo Horizonte-MG: prevalence and characterization of isolates. J Bras Patol Med Lab 2013;49:34-8.
25. Wiegering V, Kaiser J, Tappe D, Weißbrich B, Morbach H, Girschick HJ. Gastroenteritis in childhood: a retrospective study of 650 hospitalized pediatric patients. Int J Infect Dis 2011;15:e401-e7.
26. Rizi KS, Najar-Peerayeh S, Bakhshi B, Rahbar M. Prevalence of ESBLs and Integrons in Clinical Isolates of Salmonella spp. From Four Hospitals of Tehran. Int J Enteric Pathog 2015;3.
27. Shrestha KL, Pant ND, Bhandari R, Khatri S, Shrestha B, Lekhak B. Re-emergence of the susceptibility of the Salmonella spp. isolated from blood samples to conventional first line antibiotics. Antimicrob Resist Infect Control 2016;5:1.
28. Garg N, Tewari R, Gupta V, Kapil A, Kumar S, Rishi P. Current antibiogram and clonal relatedness among drug-resistant Salmonella enterica Serovar Typhi in Northern India. Microb Drug Resist 2013;19:204-11.
29. Chand HJ, Rijal KR, Neupane B, Sharma VK, Jha B. Re-emergence of susceptibility to conventional first line drugs in Salmonella isolates from enteric fever patients in Nepal. J Infect Dev Ctries 2014;8:1483-7.
30. Firoozeh F, Shahcheraghi F, Salehi TZ, Karimi V, Aslani M. Antimicrobial resistance profile and presence of class I integrongs among Salmonella enterica serovars isolated from human clinical specimens in Tehran, Iran. Iran J Microbiol 2011;3:112-7.
31. Sjölund-Karlsson M, Howie RL, Crump JA, Whichard JM. Fluoroquinolone susceptibility testing of Salmonella enterica: detection of acquired resistance and selection of zone diameter breakpoints for levofloxacin and ofloxacin. J Clin Microbiol 2014;52:877-84.
32. Ebner P, Garner K, Mathew A. Class 1 integrons in various Salmonella enterica serovars isolated from animals and identification of genomic island SGI1 in Salmonella enterica var. Meleagridis. J Antimicrob Chemother 2004;53:1004-9.
33. Eshraghi S, Dalall S, Mehdi M, Fardsanei F, Zahraii Salehi T, Ranjbar R, et al. Salmonella enteritidis and antibiotic resistance patterns: a study on 1950 children with diarrhea. Tehran Univ Med J 2010;67:876-82.
34. Salehi TZ, Mahzounieh M, Saeedzadeh A. The isolation of antibiotic-resistant Salmonella from intestine and liver of poultry in Shiraz province of Iran. Int J Poultry Sci 2005;4:320-2.
35. Lindstedt B-A, Heir E, Nygård I, Kapperud G. Characterization of class I integrons in clinical strains of Salmonella enterica subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. J Med Microbiol 2003;52:141-9.
36. Cabrera R, Marco F, Vila J, Ruiz J, Gascón J. Class 1 integrons in Salmonella strains causing traveler's diarrhea. Antimicrob Agents Chemother 2006;50:1612-3.
37. Jin Y, Ling J. Prevalence of integrons in antibiotic-resistant Salmonella spp. in Hong Kong. Jpn J Infect Dis 2009;62:432-9.
38. Van TTH, Moutafis G, Tran LT, Coloe PJ. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. J Appl Environ Microbiol 2007;73:7906-11.
39. Su L-H, Wu T-L, Chia J-H, Chu C, Kuo A-J, Chiu C-H. Increasing ceftriaxone resistance in Salmonella isolates from a university hospital in Taiwan. J Antimicrob Chemother 2005;55:846-52.
40. Li W-C, Huang F-Y, Liu C-P, Weng L-C, Wang N-Y, Chiu N-C, et al. Ceftriaxone resistance of nontyphoidal Salmonella enterica isolates in Northern Taiwan attributable to production of CTX-M-14 and CMY-2 β-lactamases. J Clin Microbiol 2005;43:3237-43.
41. Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, et al. Emergence of domestically acquired ceftriaxone-resistant Salmonella infections associated with AmpC β-lactamase. Jama 2000;284:3151-6.
42. Winokur P, Canton R, Casellas J-M, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region. Clin Infect Dis 2001;32:S94-S103.
43. Rotimi VO, Jamal W, Pal T, Sovenned A, Albert MJ. Emergence of CTX-M-15 type extended-spectrum β-lactamase-producing Salmonella spp. in Kuwait and the United Arab Emirates. J Med Microbiol 2008;57:881-6.
44. Moubareck C, Doucet-Populaire F, Hamze M, Daoud Z, Weill F-X. First extended-spectrum-β-lactamase (CTX-M-15)-producing Salmonella enterica serotype Typhimurium isolate identified in Lebanon. Antimicrob Agents Chemother 2005;49:864-5.