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

Pediatric İshal Örneklerinden Toplanan Salmonella Türlerinde İntegran Tiplerinin ve Genişletilmiş Spektrumu B-Laktamaz Genlerinin PCR Tabanlı Tanılanması

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Abstract

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.

Methods: 405 non-duplicate 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 integrons 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.

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INTRODUCTION

Salmonella spp. are recognized as major food-borne pathogens 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 primarily 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 sulhydryl 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 to integrate (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, aminoglycoside modifying enzymes (AMEs) (17, 18). Class II Integron located on 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-duplicate 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 ofoxacin (OFX; Sug) (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 (blaTEM, blaCTX-M and blaSHV) 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 (blaTEM, blaCTX-M and blaSHV) 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 10X PCR buffer, 1.5 μl MgCl2 (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 minute at 95°C, 1 minute at 65°C, and 1 minute at 72°C; and one cycle of 10 minutes at 72°C. Briefly, the whole 25 μl volume of PCR mixture reaction mixture was positive for the 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.

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%). 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 blaTEM, 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 \textit{blaSHV} gene was not identified in any of the \textit{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 \textit{S. Enteritidis} (n; 13, 56.5\%) followed by \textit{S. Infantis} (n; 6, 26\%) and \textit{S. Typhimurium} (n; 4, 17.5\%). So, 6 strains including, 4 (66.7\%) \textit{S. Enteritidis} and 2 (33.3\%) \textit{S. Infantis} carried class II integron. No class III integron-positive isolates were detected by M-PCR analysis. In the present study, \textit{S. Bardo} and \textit{S. Heidelberg} were negative for \textit{int}, \textit{intI and intII} and EÇBLs 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, cefazidime; 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 of ESBLs and integron genes among \textit{Salmonella} spp., isolated from pediatric feces samples. In total, 54 \textit{Salmonella} spp., strains were analyzed throughout the project. Our prevalence of \textit{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 \textit{S. Enteritidis} strains in pediatric stool samples (n; 24, 44.5\%), these findings were agreement with previous reports (21, 23). In a study conducted by Abdullahi et al (21), 94.2\%, 72.8\%, 31.8\%, 22.2\% resistant to the CRO, IPM and OFX were observed in \textit{Salmonella} spp., strains were analyzed throughout the project. Our prevalence of \textit{Salmonella} spp., in stool samples was obtained 13.3\%.

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### CONCLUSION

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

### Conflict of interest

No conflict of interest was declared by the authors.

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### REFERENCES

1. McEvoy J, Doherty A, Sheridan J, Blair I, McDowell D. The prevalence of \textit{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, Pournaajaf A, Gholami M, Talebi M. Detection of New Virulence Genes in mecA-positive \textit{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 \textit{Salmonella} gastroenteritis. Clin Infect Dis 2010;50:882-9.
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-Saraye 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-ACCSt Salmonella Typhimurium and MDR-AmpC Salmonella Newport in the United States, 2003-2005. Foodborne Pathog Dis 2008;5:669-80.

9. Nkaido 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, Mehrtra M, Ghimire S, Adewoeye 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, Khadka 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 a new safer 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, Cooes 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 nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

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. Smolinka A, Lestâr B, Fekete PZ, Nagy B. Conjugative InCl and InCl1 plasmids with tet (A) and class 1 integron conferring multidrug resistance in F18+-producing Salmonella enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for a new safer alternatives. Int J Infect Dis 2006;10:434-8.

18. Forrest PW, 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.

19. Greene SK, Stuart AM, Medalla FM, Whichard JM, Hoekstra RM, Chiller TM. Distribution of multidrug-resistant human isolates of MDR-ACCSt Salmonella Typhimurium and MDR-AmpC Salmonella Newport in the United States, 2003-2005. Foodborne Pathog Dis 2008;5:669-80.

20. Serotypes in Katsina State, Nigeria. Afr J Microbiol Res 2014;8:915-6.

21. Koizumi T, Aoki S, Oshita S, Tanihata I, Kato M. Prevalence of class 1 integrons and antibiotic resistance among Escherichia coli isolates from a university hospital in Taiwan. J Antimicrob Chemother 2005;55:846-850.

22. Dunne EF, Fey PD, Kludt P, Reporter R, Mostashari F, Shillam P, et al. Emergence of domestically acquired ceftriaxone resistance of nontyphoidal Salmonella enterica isolate S103. Jpn J Infect Dis 2009;62:432-9.

23. Eshagi S, Danzil S, Mehdi M, Fardiansi F, Zahraei Salehi T, Ranjbar, et al. Salmonella enteritidis and antibiotic resistance patterns: a study on 1950 children with diarrhea. Tehran Univ Med J 2010;67:876-82.

24. Salehi TZ, Mahzouneh M, Saeedazadeh A. The isolation of antibiotic-resistant Salmonella from intestine and liver of poultry in Shiraz province of Iran. Int J Poult Sci 2005;4:320-2.

25. Vo AT, Van Duijkeren E, Fluit AC, Wannet WJ, Verbruggen AJ, Maas HM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

26. 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.

27. Smolinka A, Lestâr B, Fekete PZ, Nagy B. Conjugative InCl and InCl1 plasmids with tet (A) and class 1 integron conferring multidrug resistance in F18+-producing Salmonella enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for a new safer alternatives. Int J Infect Dis 2006;10:434-8.

28. Forrest PW, 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.

29. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

30. Vo AT, Van Duijkeren E, Fluit AC, Wannet WJ, Verbruggen AJ, Maas HM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

31. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

32. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

33. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

34. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

35. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

36. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

37. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

38. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

39. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.

40. Cho K, Choi JY, Lee JW, Kim SC, Park J, Lee SM, et al. Antibiotic resistance, integrons and Salmonella genomic island 1 among nontyphoidal Salmonella serovars in The Netherlands. Int J Antimicrob Agents 2008;26:172-9.