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Molecular Characterization of Multiple Antibiotic Resistance in *Salmonella enterica* Serovar Typhimurium and Eenteritidis Isolated in Saudi Arabia

Eman Halawani and Mohamed Shohayeb

Department of Biology, Girls, Faculty of Science, Taif University, Saudi Arabia

Abstract: Twenty-two Salmonella strains isolated from acute salmonella food poisoning were investigated in this study. The isolates were 17 *Salmonella enterica* Serovar Typhimurium and 5 *S.* Enteritidis. Salmonella isolates were tested for their susceptibility to ten commonly used antibiotics. All isolates were resistant to 2-5 antibiotic and it was possible to recognize five phenotypic resistance profiles in serotype Typhimurium and two in serotype Enteritidis. Sixty-eight % of isolates were resistant to 4 or 5 antibiotics. Resistance determinants were checked whether they were carried on plasmids or chromosomally as in the case of the world wide prevalent DT104 strain of *S.* Typhimurium. All investigated salmonella strains harbored 2-6 plasmids ranging in their molecular weights from 3.8 to 90 kb in *S.* Typhimurium and 4 to 65 kb in *S*. Enteritidis. Conjugation studies revealed that although not all plasmids were transferable by conjugation, all resistance determinants were transferable to sensitive recipient stain of *E. coli* k12. Both high and low molecular weight plasmids contributed in the transfer of resistance determinanats to recipient E. coli strain. This suggests that these resistance determinants are carried extra-chromosomally on R-plasmids and rules out the prevalence of the multi-drug resistant *S.* Typhimurium strain DT104 which has been reported in different countries including some gulf countries. Data found in this study suggest a misuse of antibiotics, a fact that necessitate the control of antibiotic use in Saudi Arabia both in humans and animals.

Key words: Molecular characterization · *Salmonella enterica* · Saudi Arabia

INTRODUCTION

Salmonella food poisoning is one of the most common and widely distributed diseases in the world [1-3]. Outbreaks are usually associated with ingestion of contaminated food of animal origin like, poultry, meat and milk [4,5]. Although the majority of infections result in asymptomatic or self-limited disease, in immuno-compromised patients, neonates and elderly antibiotic treatment is usually recommended [6,7]. It is estimated that the annual economic costs due to food-borne salmonella infections in the United States are $2.4 billion [8].

Recently multi-drug resistant (MDR) strains, have emerged, presumably due to the extensive use of antimicrobial agents both in humans and animals [9,10]. In veterinary medicine antibiotics are used in livestock production, disease prevention and as growth-promoting feed additives [4,11]. The use of antibiotics in animals disrupts the normal flora of the intestine, resulting in the emergence of antibiotic-resistant salmonella strains and prolonged fecal shedding of these organisms into the environment [12,13]. MDR in salmonella is a cause of great concern in both clinical and veterinary medicine, because it may limit the therapeutic options available for their treatment [14,15]. The fatality rate for people infected with antibiotic-resistant salmonella strains is 21 time greater than for individuals infected with non-antibiotic resistant salmonella strains [16].

Resistance markers may be carried on plasmids or the chromosome of resistant bacteria [17]. Plasmids have been a major factor in the spreading antibiotic resistance between bacteria [18,19]. Under antibiotic selective pressure, R-plasmids spread resistance markers between both homologous and heterogonous bacterial communities [20]. Recently, an epidemic multi-drug resistant strain serovar Typhimurium phage type 104 (DT104) has been identified as a major cause of salmonellosis in humans and animals, in both Europe and the United States [14,17,21]. Resistance genes of DT104 isolates are located within certain locus on the chromosome and they confer resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline.

Corresponding Author: Dr. Mohamed Shohayeb, Department of Biology, Girls, Faculty of Science, Taif University, Saudi Arabia

In a previous study, we demonstrated the prevalence of multi-drug resistant *S. enterica* isolated from both infected cases and carriers [22]. In this study we characterize whether antimicrobial resistance genes in serovars Typhimurium and Enteritidis are located on plasmids or the chromosome. This would help to understand their diversity and their ease of dissemination in the Saudi community.

METHODS

Bacteria: Salmonella strains were isolated from patients suffering from acute food poisoning admitted to King Abdul Aziz and Al-Noor hospitals in Taif and Makkah Al- Mukaramah respectively, during the summer of 2007. Identity of collected strains was confirmed biochemically using API 20E kits (Bio Merieux, France). Serotypes were determined by slide agglutination test using specific antisera (Meurex Biotech Ltd, UK). For conjugation studies a standard *Escherichia coli* K12 ^{NxR} strain (UB 5202), resistant to nalidixic acid was used as a recipient strain.

Antibiotic susceptibility: Salmonella isolates were tested for their susceptibility to eleven antibiotics by disc diffusion method on Muller-Hinton agar (Oxoid, UK), according to the recommendations of National Committee for Clinical Laboratory Standards [23]. The antibiotics used were ampicillin (Ap, 10 μg), cephalothin (Cl, 30 μg); cefotaxime (Cf, 30 μg); Streptomycin (Sm, 10 μg); gentamicin (Gm, 10 μg); amikacin (Ak, 30 μg); tetracycline (Tc μg); chloramphenicol (Cm, 10 μg); ciprofloxacin (Cp, 1 μg) and sulfamethoxazole (Sx, 250 μg).

Conjugation experiments: Conjugation was carried out as previously describe [24]. Briefly both donor salmonella isolates and recipient *E. coli* cells were grown to logarithmic phase, mixed together and plated onto nutrient agar plates. Conjugation was allowed to take place for 48 h at 37°C. The mating cells were subcultured onto nutrient agar plates containing 30 μg nalidixic acid plus one of the antibiotics to which the donor strain was resistant. Antibiotics used for selection of transconjugants were Amp (50 μg/ml), Tet (25 μg/ml), Clm (20 μg/ml), Stm (50 μg/ml) and Sul (200 μg/ml) and. Transconjugants were tooth picked onto series of plates containing nalidixic acid plus one of the antibiotics mentioned above to check the co-transfer of resistance determinants.

Isolation of Plasmids: Plasmid DNA was extracted from bacterial cells grown overnight in 1.5 ml nutrient broth. Small scale alkaline lyses method was used as described by Sambrook, *et al.,* [26]. Isolated plasmids were electrophoresed in 0.8% horizontal agrose gel using a Bio-Rad Sub Cell horizontal electrophoresis apparatus (Bio-Rad laboratories, Richmond, Calif) Electrophoresis was carried out at 20 mA for about 18 h. DNA was stained by ethidium bromide (0.5 μg/ml) and bands were visualized by U.V. transilluminator (Fisher, UK). Photographs were taken by a polaroid camera using 667 instant films (Polaroid incorporation, UK). The molecular sizes of plasmids were estimated by comparison to plasmids of known molecular sizes run on the same gel.

RESULTS

Twenty-two salmonella strains isolated from acute salmonella food poisoning were included in this study. The isolates were 17 *S.* Enterica Typhimurium and 5 *S.* Enteritidis. Isolates were tested for their susceptibility to Ap, Cl, Cf, Tc, Cm, Sm, Ak, Gn, Sx and Cp. All isolates were susceptible to Cf, Cp, Ak and Gn. Resistance to other antibiotics ranged between 30-79% (Data not shown).

While, *S.* Typhimurium isolates were resistant to 3-5 antibiotics, *S.* Enteritidis were resistant to 2-4 antibiotics (Table 1). *S.* Typhimurium isolates were categorized into five phentype groups according to their resistance profile to antibiotics. Two resistance phentotypes were detected in of *S.* Enteritidis (Table 1). Resistance patterns; Ap Sm Sx Tc and Ap Sm Tc Sx Cm, were more common, being detected in 9 and 7 isolates respectively, of the 22 tested (Table 1).

All isolates harbored between 2-6 plasmids with molecular sizes ranging between 4 and 90 Kb (Fig. 1 and Table 1). The total number of plasmid profiles was 9 profiles (Table 1). Some isolates shared the same resistance patterns, yet, they had different plasmid contents. This was observed in resistance phenotypes I and II. For example, the plasmid profile for the resistance pattern, Ap Sm Tc Sx Cm (phenotype I) found in *S.* Typhimurium had plasmids of sizes 90, 20.4, 17.8, 7.9, 6.0 or 90, 20.4 kb (Table 1). This phenomenon was not observed in *S.* Enteritidis isolates. The molecular sizes of *S.* Typhimurium plasmids ranged between 3.8 and 90 Kb while in *S.* Enteritidis the molecular size ranged between 4 and 65 Kb. (Fig. 1 and Table 1). The high molecular weight plasmids, 90 kb was observed in all tested

Table1: Resistance patterns and plasmid contents of some salmonella isolates and *E. coli* K12 transconjugants

Donor resist. gp	No isolates	R-pattern	Plasmid profile	R-gene selected	Co-transferred R-genes	Conjug. frequency	Transconjugants resist. pattern	Plasmids tansferred
I	4	Ap Sm Tc Sx Cm	90, 20.4, 17.8, 7.9, 6.0	Ap	Sm Tc Sx Cm	$1X10^{-8}$	Ap Sm Tc Cm	90, 20.4
				Tc	Sm Sx Cm Ap	$4X10^{-10}$	Ap Sm Tc Cm	
				Cm	Sx	$2X10^{-9}$	Sx Cm	
				Sx	Cm	$3X10^{-8}$	Sx Cm	
	3	Ap Sm Tc Sx Cm	90, 20.4	Ap	Sm Sx Cm	$3X10^{-9}$		
				Sm	Ap Sx Cm	$2X10^{-8}$	Ap Sm Sx Tc Cm	90, 20.4
				Sx	Cm	$2X10^{-8}$		
				Cm	Sx	$4X10^{-8}$		
II	4	Ap Sm Sx Tc	90, 20, 7.9, 6	Sx	ND	$2X10^{-8}$	Sx	90, 20
	2	Ap Sm Sx Tc	90, 20, 17.8, 7.9, 6	Ap	Sm Tc Sx	$4X10^{-9}$	Ap Sm Tc Sx	90, 20, 7.9
				Sm	Ap Tc Sx	$2X10^{-8}$		
				Tc	Ap Sx Sm	$4X10^{-9}$		
III	2	Ap Sm Sx	90, 20.4, 7.9, 6.0	Ap	Sm Sx	$9X10^{-9}$	Ap Sm Sx	90, 20.4, 7.9
				Sm	Apsx	$4X10^{-9}$		
				Sx	Ap Sm	$3X10^{-8}$		
IV	2	Sm Tc Sx	90, 28.2, 20,15.8, 9.1, 3.8	Sx	ND	$3X10^{-8}$	Sx	20
V	3	Ap Sm Tc Sx	65, 20.6, 4	Ap	Sm Tc Sx	$2X10^{8}$	Ap Sm Tc Sx	65, 20.6
				Tc	Ap Sm Sx	7X109		
				Sm	Ap Tc Sx	$3X10^{-8}$		
				Sx	Ap Sm Tc	$1X10^{-8}$		
VI	2	Sm Sx	65, 20.6	Sm	Sx	$2X10^{-7}$	Sm Sx	65
				Sx	Sm	$8X10^{-7}$		

Table 2: Transfer of different plasmids of 22 salmonella isolates

Donor	Plasmid (kb)	Incidence (%)	No. of trans- conjugants(%)
S. Typhimurium	90	17 (100)	17 (100)
	28.2	2 (11.8)	ND
	20.4	9 (52.9)	9 (100)
	20.0	6 (35.3)	6 (100)
	17.8	2 (11.8)	ND
	9.1	2 (11.8)	ND
	7.9	10 (58.8)	2 (20)
	6.0	12 (70.6)	ND
	3.8	2 (11.8)	ND
S. Enteritidis	60.0	5 (100)	5 (100)
	20.6	5 (100)	5 (100)
	4.0	2 (40)	ND

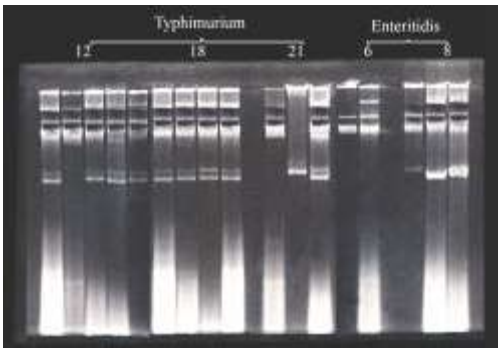

Fig. 1: Plasmid profile of *S. enterica* isolates and their *E. coli* transconjugants

S. Typhimurium and the high molecular weight 65 kb was detected in all tested and *S.* Enteritidis (Fig 1 and Table 1,2). Plasmids of molecular sizes 6.0 kb, 7.9 kb and 20.4 kb were more common *S.* Typhimurium and were detected in 70.6, 58.8 and 52.9% of isolates (Table 2).

The 22 isolates were tested for their ability to transfer their plasmid contents to a standard a recipient *E. coli* K12 strain (Fig. 1 and Table 1). Out of the 9 plasmids detected in different *S.* Typhimurium isolates only 4 (90.0 kb, 20.4 kb, 20.0 kb and 7.9 kb), were transferable (Table 2). Three plasmids of these plasmids were transferable in all conjugation studies while one (7.9 Kb) was transferable in only 20% of conjugation studies.

Several resistance determinants used to be co-transferred in each conjugation experiment to the *E. coli* standard strain (Tables 1 and 3). The rate of

Table 3: Transfer of antibiotic resistance markers and cotransferable markers in salmonella isolates

Donor	Resist marker	No. of resist isolates	Incidence of marker transfer (%)	Co-transfered markers (No).
*S.*Typhimurium	Ap	15	11 (73.3)	Tc(6), Cm(7), Sm(6), Sx(11)
	Cm	7	7 (100.0)	Tc(4), Ap(7), Sm(7), Sx(3)
	Tc	13	6 (46.2)	Cm(4), Ap(6), Sm(6), Sx(2)
	Sm	17	11 (64.7)	Cm(7), Ap(11), Tc(6), Sx(11)
	Sx	17	15 (88.2)	Cm(3), Ap(7), Tc(2), Sm(11)
*S.*Enteritidis	Ap	3	3 (100.0)	Tc(3), Sm(3), Sx(3)
	Tc	3	3(100.0)	Cm(3), Sm(3), Sx(3)
	Sm	5	5(100.0)	Tc(3), Ap(3) Sx(5)
	Sx	5	5(100.0)	Tc(3), Ap(3), Sm(5)

conjugation ranged between $4X10^{-10}$ to $2X10^{-7}$ (Table 1). The antibiotics with highest incidence of co-transfer in *S.* Typhimurium with Ap was Sm; with Cm was Ap and Sm; with Tc was Ap and Sm; with Sm was Ap and Sx; and with Sx was Sm (Table 3).

The High molecular weight plasmids (90 kb and 65 kb) in both serogroups were associated with transfer of resistance determinants of several antibiotics like ampicillin, streptomycin, tetracycline sulfonamide and chloramphenicol (Table 1). The plasmid 20.4 was associated with transfer of resistance markers of Cm and Sx and the plasmid 20 kb was associated with the transfer of resistance determinants of Sx. In 4 isolates resistance determinants of Ap, Sm and Tc were chromosomal and were not transferable by conjugation (Table 1).

DISCUSSION

There has been an increasing incidence of antibiotic resistance in salmonella isolates which has been documented worldwide [11,15,26] and locally in Saudi Arabia [22,27,28]. This increase is attributed to the selection pressure created by uncontrolled use of antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics by humans [29,30,31].

The lack of stringent controls on antimicrobial usage increases the risk of dissemination of food-borne microbes harboring arrays of resistance genes. The objective of this study was to determine the prevalence of resistant *Salmonella* spp., isolated from acute cases of food poisoning and characterize whether resistance determinants were located on the chromosome or plasmids. This would help to understandthe diversity of MDR strains and their potential dissemination.

All isolates were resistant to 2-5 antibiotic and it was possible to recognize five phenotypic resistance profiles in *S.* Typhimurium and two in *S.* Enteritidis. Sixty-eight % of isolates were resistant to 4 to 5 antibiotics. This demonstrates the prevalence of MRD salmonella in Taif and reflects uncontrolled use of antibiotics in the Society. Plasmid profiles revealed that bacterial isolates with the same resistance profile may differ in their plasmid profiles. This suggests a diversity in plasmid contents of bacterial isolates and the contribution of different plasmids in the resistance to a certain antibiotic.

MRD salmonella isolates have been reported since the 1960s [32], with the resistance patterns of *Salmonella* serovars of public health importance often associated with specific phage types. One notable MDR strain is *S. enterica* serovar Typhimurium DT193 which carries its resistance determinants on conjugative plasmids [10,33,34]. Another type DT104 strains are commonly known to be penta-resistant, exhibiting resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (the AmCmStSuTe resistance phenotype). Antimicrobial resistance genes were found to be clustered on the chromosome of *S.* Typhimurium DT104 [35-37]. The resistance pattern of DT104 was found in 7 isolates of the 22 examined in this study, which suggested a possibility for theses isolates to be of the same phage type. However, conjugation analysis revealed that although not all plasmids were transferable by conjugation, all resistance determinants were transferable to sensitive recipient stain of *E. coli*. This suggests that these resistance determinants were carried extra-chromosomally on R-plasmids and rules out the presence of strain DT104. This is surprising because the kingdom imports live and slaughtered animals from abroad and there is a global dissemination of DT104 in different countries like United States, South Africa, the United Arab Emirates, Trinidad, the Philippines, the Irish Republic, Canada, Israel, Japan, Vietnam, Taiwan and most European countries [11,13,21,38-40].

All strains harbored 2-6 plasmids ranging in their molecular weights from 3.8 to 90 kb in *S.* Typhimurium and 4 to 65 kb in *S.* Enteritidis. Large plasmids are believed to be responsible for pathogenicity of the bacteria and at the same time have a role in MRD of salmonella [10]. Large plasmids expressed resistance to several antibiotics including Ap, Cm, Sm, Sx and Tc [41]. Multiple resistance genes clusters in large plasmids are usually associated with transposons and insertion sequences [10]. Conjugation studies suggest that 90 kb plasmid contributes in resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline as found before [41]. On the other hand low molecular weight

plasmids were also found to be responsible for resistance to antibiotics [42]. A plasmid of a size of 20.4 was found to be likely responsible for resistance to Cm and Sx.

It may be concluded that MRD *S.* Typhimurium and Enteritidis are prevalent in the Taif community. Resistance determinants of the 22 investigated isolates were found to be located on plasmids, nevertheless this does not negates the possibility that *S.* Typhimurium DT104 could be detected if a larger sample of isolates is investigated. It should be mentioned that plasmids spread very fast in both homologous and heterologous bacterial communities [20], a fact that necessitate the control of antibiotic use in Saudi Arabia both in humans and animals

REFERENCES

1. Rodrigues, D.C., R.V. Tauxe and B. Rowe, 1990. International increase in Salmonella enteritidis: A new pandemic? Epidemiol Infect, 105:21-7.
2. Gomez, T.M., Y. Motarjemi, S. Miyagawa, F.K. Kaferstein and K. Stohr, 1997. Foodbome salmonellosis. World Health Stat Q., 50: 81-9.
3. WHO, 2005. http://www.who.int/mediacentre/factsheets/fs139/en.
4. Swartz, M.N., 2002. Human diseases caused by food-borne pathogens of animal origin. Clin. Infect. Dis., 34(Suppl 3): S111-22.
5. Lazaro, N., A. Tibana, D.P. Rodrigues, E. Reis, Br. Quintaes and E.Hofer, 2004. Antimicrobial resistance and R-plasmids in *Salmonella* spp. From swine and abattoir environment. Pesq Vet Bras, 24: 57-60.
6. Chalker, R.B. and M.J.1. Blaser, 1988. A review of human salmonellosis: III. Magnitude of *Salmonella* infection in the United States. Rev. Infect Dis., 10: 111-24.
7. Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, 1999. Food-related illness and death in the United States. Emerg Infect Dis., 5: 607-25.
8. Gebreyes, W.A. and C. Altier, 2002. Molecular Characterization of Multidrug-Resistant *Salmonella enterica* subsp. *enterica* Serovar Typhimurium Isolates from Swine J. Clin. Microbiol., 40: 2813-2822.
9. Murray, B.E., 1986. Resistance of shigella, salmonella and other selected enteric pathogens to antimicrobial agents. Rev Infect Dis, 8 (Suppl. 2): S172-S181.
10. Miriagou, V., A. Carattoli and S. Fanning, 2006. Antimicrobial resistance islands: resistance gene clusters in Salmonella chromosome and plasmids. Microbes Infect, 8: 1923-1930.
11. Van, T.T.H., G. Moutafis, T. Istivan, L.T. Tran and P.J. Coloe, 2007. Detection of *Salmonella* spp. in Retail Raw Food Samples from Vietnam and Characterization of Their Antibiotic Resistance, Appl. Environ. Microbiol., 73: 6885-6890.
12. Sun, M., 1984. In search of *Salmonella's* smoking gun. Science, 226: 30-32.
13. Threlfall, E.J., 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol. Rev., 26: 141-148.
14. Glynn, M.K., C. Boppy, W. Dewitt, P. Dabney, M. Mokhtar and F.J. Angulo, 1998. Emergence of multi-drug resistant *Salmonella enterica* serovar Typhimurium DT104 infections in the United States. N. Engl. J. Med., 338: 1333-1338.
15. Carattoli, A., E. Filetici, L. Villa A.M. Dionisi A. Ricci and I. Luzzi, 2002. Antibiotic resistance genes and *Salmonella* genomic island 1 in *Salmonella enterica* serovar Typhimurium isolated in Italy. Antimicrob Agents Chemother, 46: 2821-2828.
16. Helms, M., P. Vastrup, P. Gerner-Smidt and K. Molbak, 2003. Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. BMJ., 326: 357.
17. Tauxe, R., 1999. Salmonella Enteritis and Salmonella Typhimurium DT104. Successful subtypes in the modern world. In: Emerging infections. Washington: ASM Press, pp: 37-52.
18. Davis, J., 1994. Inactivation of antibiotics and dissimination of resistance genes. Sci., 264: 375-382.
19. Chu, C., C. Chiu, W. Wu, C. Ch, T. Liu and J. Ou, 2001. large drug resistance virulence plasmids of clinical isolates of *Salmonella enterica* serovar Coleaesuis. Antimicrob Agents Chemother, 54: 2299-2203.
20. Dionisio, F., I. Matic, M. Ramasan, O.R. Rodrigues and F. Taddei, 2002. plasmid spread very fast in heterologous bacterial communities. Genetics, 162: 1525-1532.
21. Besser, T.E., M. Goloft, L.C. Pritcheltt, R. Khakharia, D.D. Hankock, D.H. Rices, J.M. Gay, W. Johnson and C.C. Gay, 2000. Multiresistant Salmonella Typhimurium DT104 infection in humans and domestic animals in the Pacific Northwest of the United States. Epidemiol. Infect., 124: 193-200.
22. Halwani, E. and M. Shohayeb, 2008. Salmonella food poisoning in Taif, Saudi Arabia. Under publication.

23. National Committee for Clinical Laboratory Standards, 2003. Performance standards for antimicrobial disc susceptibility tests. M2A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
24. Shohayeb, M. and F. Sonbol, 1994. Conjugal transfer of antibiotic resistance of shigella isolated in Tanta. Alex. J. Pharm. Sci., 8: 177-180.
25. Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, 2nd edition, New York.
26. Threlfall, E.J., L.R. Ward, J.A. Frost and G.A. Willshaw, 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. Int. J. Food Microbiol., 62: 1-5.
27. Kambal, A.M., M.N. Chowdhury, Y.A. Al-Eissa, F.A. Al-Zamil and M.A. Al-Kharashi, 1992. Salmonella gastroenteritis in children: experience at a teaching hospital in Riyadh, Saudi Arabia. Med. Sci. Res., 20: 149-151.
28. Malik, G.M., A.H. Al-Wabel, M.M. El-Bagir, K. Ahmed, N.F. Bilal and A. Shenoy, 1993. Salmonella infection in Asir region southern Saudi Arabia: expatriate implications. Ann. Saudi Med., 13: 242-245.
29. Aarestrup, F.M., 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Intl. J. Antimicrob Agents, 12: 279-285.
30. Van den Bogaard A.E. and E.E. Stobberingh, 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Intl. J. Antimicrob, Agents, 14: 327-335.
31. Teuber, M., 2001. Veterinary use and antibiotic resistance. Curr Opin Microbiol., 4: 493-499.
32. Anderson, E.S. and M.J. Lewis, 1965. Drug resistance and its transfer in *Salmonella typhimurium*. Nature, 206: 579-583.
33. Hampton, M.D., E.J. Threlfall, J.A. Frost, L.R. Ward and B. Rowe, 1995. *Salmonella typhimurium* DT193: differentiation of an epidemic phage type by antibiogram, plasmid profile, plasmid fingerprint and salmonella plasmid virulence (spv) gene probe. J. Appl. Bacteriol., 78: 402-408.
34. Varteresian, I.I., Z.U. Gevorkian, N.N. Sarkisian, A.M. Sedrakian, K.A. Arakelova, Zh. A. Ktsoyan, 2002. Conjugative plasmids of Salmonella resistant to antibiotics. Zh Mikrobiol Epidemiol. Immunol., 1: 78-79.
35. Ridley, A. and E.J. Threlfall, 1998. Molecular epidemiolofy of antibiotic resistance genes in multiresisant epidemic Salmonella Typhimurium DT401. Microb Drug. Res., 4: 113-118.
36. Briggs, C.F. and P.M. Frtamico, 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella Typhimurium* DT104. Antimicrob Agents Chemother, 43: 846-849.
37. Levings, R.S., D. Lightfoot, S.R. Partridge, R.M. Hall and S.P. Djordjevic, 2005. The Genomic Island SGI1, Containing the Multiple Antibiotic Resistance Region of *Salmonella enterica* Serovar Typhimurium DT104 or Variants of It, Is Widely Distributed in Other *Salmonella enterica* Serovars. J. Bacteriol., 187: 4401-4409.
38. Ribot, E.M., R.K. Wierzba, F.J. Angulo and T.J. Barrett, 2002. *Salmonella enterica* serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990 and 1995. Emerg. Infect Dis., 8: 387-391.
39. Faldynova, M., M. Pravcoca, F. Sisak, H. Havlickova, I. Kolackova, A. Cisek, R. Karpiskova and I. Rychlik, 2003. Evolution of antibiotic of antibiotic resistance in Salmonella enterica Servar Typhimurium strains isolated in the Czech Republic. Antimicrob Agents Chemother., 47: 2002-2005.
40. Chiu, C., L. Su, C. Chu, M. Wang, C. Yeh, F. Weill and C. Chu, 2006. Detection of Multidrug-Resistant *Salmonella enterica* Serovar Typhimurium Phage Types DT102, DT104 and U302 by Multiplex PCR. J. Clin. Microbiol., 44: 2354-2358.
41. Guerra, B., S.M. Soto, A.J.M. Rguelles and M.C. Mendoza, 2001. Multidrug resistance is mediated by large plasmids carrying a class 1 integron in the emergent *Salmonella enterica* serotype [4,5,12:i]. Antimicrob Agents Chemother, 45: 1305-1308.
42. Simmon, K.W., R.E. Wooley and J. Brown, 1988. Comaparison of virulence factors and R-plasmids of *Salmonella* spp isolated from healthy and ill swine. Appl. Environ. Microbiol., 54: 760-767.