Original Research Article

Antibiotic resistance pattern and frequency of SHV, CTX, TEM, and OXA resistance gene among *salmonella* serotypes

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ABSTRACT

The aim of this study was to investigate the antibiotic resistance and identification of strains producing broad-spectrum beta-lactamases *salmonella* strains. The 70 different strains of *salmonella* were studied that gentamicin, ciprofloxacin, ofloxacin, imipenem, and enrofloxacin were susceptible. The highest resistance to cephalexin (96 %), cefazolin (96 %), cephalothin (65 %), and 69 isolates (98 %), were resistant to more than one antibiotic. The results of the Polymerase Chain Reaction (PCR) were evaluated on ESBL producing isolates using specific primers; in order to investigate the presence of CTX-Mbla, SHVbla, TEMbla and OXAbla, so the presence of germ cell resistance genes in total indicated that 32 isolates (56 %), at least one of the above genes.

***Keywords*:** Salmonella, antibiotic resistant, SHV, TEM, OXA, CTX gene

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INTRODUCTION

Salmonellosis is one of the most important infectious diseases between humans and animals. This infectious disease is caused by the *salmonella* transmitted through the food chain [1]. The microorganism is one of the major causes of digestive diseases worldwide. *Salmonella* infection can cause gastroenteritis, intestinal fever, and septicemia with local lesions. The infection is accompanied by symptoms such as fever, diarrhea, and abdominal pain and often takes 4 - 9 days.

Salmonella has two important species involved *salmonella enterica* and *salmonella benguri*. The *enterica* has six sub-species, including *salmonella enterica subsp arizonae*, *salmonella enterica subsp Enterica*, *salmonella enterica* subsp *Diarrhiza*, *salmonella enterica subsp Salami*, *salmonella enterica subsp hutana*, and *salmonella enterica subsp Indica* [2].

Antibiotic resistance is a worldwide phenomenon that leads to the emergence of pathogens with resistance to clinically important antibiotics. Antibiotic-resistant bacteria pose a serious threat to health. It is estimated that antibiotic-resistant pathogens cause 23,000 annual deaths in the United States.

Recently, drug-resistant *salmonella* has been associated with a significant number of outbreaks in the United States. *Salmonella Urbana* spread the disease through papaya in 2017, and the isolates showed resistance to streptomycin and intermediate resistance to tetracycline.

Multidrug-resistant *salmonella* in 2015 led to severe infection in humans. The strains isolated from infected pork products were resistant to various antibiotics, including ampicillin, streptomycin, sulfisoxazole, and tetracycline [3].

Different antibiotics are used to treat *salmonella* infections, and the increasing consumption of these drugs by humans and animals, it eliminates sensitive strains and selects antibiotic-resistant strains [4].

The production of β-lactamase by the gram-negative bacterium is the main cause of resistance to lactam antibiotics. These enzymes separate the amide transplant in the β-lactam ring and make antibiotics β-lactamase safe for bacteria [1].

Extended Spectrum Beta Lactamases (ESBL) is able to hydrolyze penicillins, first, second, third, and fourth-generation cephalosporins and monobactam aztreonam. ESBLs (especially TEM and SHV family derivatives) are readily inhibited by available commercially available beta-lactamase inhibitors (clavulanic acid, tazobactam, or sulbactam). This unique property acts as an important phenotypic test that is easily performed to identify bacterial ESBL [5].

Broad-type beta-lactamases, TEM, SHV, CTX-M, PER, and OXA are the most common types of β-lactamase that have been identified so far.

This study aims to investigate the antibiotic resistance and identification of strains producing broad-spectrum beta-lactamases of type SHV, CTX-M, TEM, and OXA among *salmonella* strains.

MATERIALS AND METHODS

70 strains of *salmonella* in the microbial collections of the microbiology department of Razi Research and Certification Institute of Karaj were cultured for antibiotic susceptibility testing and the presence of CTX-M, SHV, TEM, and OXA-type genes (Table 1). A combination of selective and differential culture media was used to grow and detect the biochemical properties of *salmonella* [6].

Determination of salmonella serotypes

To determine *salmonella* serotypes, serotyping with Polyvalent O and Polyvalent H anesthetics was performed according to the Kauffman-White table. *Salmonella* serotypes were used for this purpose.

Antibiotic susceptibility test

Antibiotic resistance test was performed using a disk diffusion method based on the standard clinical practice and laboratory method [7]. The antibiotics used in this study were prepared in 50 disks of MAST and Padtanteb.

Determination of ESBL producing strains

A combined disk test was used to investigate the strains generating β-lactamase enzymes. The company's diagnostic discs (MASTs) included the following four sets (Table 2).

IF the susceptibility of antibiotic increases significantly in the presence of clavulanate (a ≥5 mm increase in a zone diameter) the result is interpreted as confirmation of ESBL production [8].

Polymerase Chain Reaction (PCR) method using specific primers was done for investigating the presence of type-1 plasmid type genes, blaCTX, blaTEM, blaSHV, and blaOXA on the isolates producing ESBL. The primers used in this study were extracted from OXA, TEM, CTX, and SHV genes from the published articles in this field, which were successfully applied by various researchers (Table 3), and were sent to Sinagen for manufacturing. Klebsiella pneumonia 600703 ATCC that carries the target gene was used as a positive control sample.

Table 1. Characteristics of the strains used in this study and the serotypes isolated

No.	*RTCC* (Razi Type Culture Collection)	Serotype	Source
1	1736	*Salmonella Urbana*	poultry
2	1737	*Victeraborg Salmonella*	poultry
3	1738	*Salmonella Enteritidis*	poultry
4	1757	*Typhimurium Salmonella*	poultry
5	1758	*Typhimurium Salmonella*	poultry
6	1759	*Typhimurium Salmonella*	poultry
7	1760	*Typhimurium Salmonella*	poultry
8	1761	*Salmonella Arizona*	poultry
9	1762	*Salmonella Arizona*	poultry
10	1763	*Salmonella Typhimurium*	poultry
11	1764	*Salmonella Typhimurium*	poultry
12	1765	*Typhimurium Salmonella*	poultry
13	1766	*Typhimurium Salmonella*	poultry
14	1767	*Salmonella Enteritidis*	poultry
15	1768	*Salmonella Enteritidis*	poultry
16	1769	*Salmonella Enteritidis*	poultry
17	1770	*Typhimurium Salmonella*	poultry
18	1771	*Typhimurium Salmonella*	poultry
19	1772	*Typhimurium Salmonella*	poultry
20	1773	*Typhimurium Salmonella*	poultry
21	1775	*Typhimurium Salmonella*	poultry
22	1776	*Typhimurium Salmonella*	poultry
23	1777	*Typhimurium Salmonella*	poultry
24	1778	*Salmonella Infantis*	poultry
25	1779	*Salmonella Congo*	poultry
26	1780	*Salmonella Infantis*	poultry
27	1781	*Salmonella Enteritidis*	poultry
28	1782	*Typhimurium Salmonella*	poultry
29	1783	*Salmonella Enteritidis*	poultry
30	1784	*Typhimurium Salmonella*	poultry
31	1785	*Salmonella Enteritidis*	poultry
32	1786	*Typhimurium Salmonella*	poultry
33	1787	*Salmonella Infantis*	poultry
34	1788	*Salmonella Typhimurium*	poultry
35	1789	*Salmonella Typhimurium*	poultry

36	1790	*Salmonella Infantis*	poultry
37	1791	*Salmonella Newport*	poultry
38	1792	*Salmonella Newport*	poultry
39	1793	*Salmonella Newport*	poultry
40	1794	*Salmonella Newport*	poultry
41	1795	*Salmonella Enteritidis*	poultry
42	1796	*Salmonella Enteritidis*	poultry
43	1797	*Salmonella Enteritidis*	poultry
44	1798	*Typhimurium Salmonella*	poultry
45	1817	*Salmonella Dublin*	poultry
46	1801	*Typhimurium Salmonella*	poultry
47	1802	*Typhimurium Salmonella*	poultry
48	1803	*Salmonella Congo*	poultry
49	1804	*Salmonella Enteritidis*	poultry
50	1805	*Salmonella Newport*	poultry
51	1806	*Salmonella Newport*	poultry
52	1807	*Salmonella II*	poultry
53	1808	*Salmonella Enteritidis*	poultry
54	1809	*Salmonella Infantis*	poultry
55	1810	*Salmonella Muenchen*	poultry
56	1811	*Salmonella Enteritidis*	poultry
57	1812	*Typhimurium Salmonella*	poultry
58	1813	*Salmonella* Gallinarum	poultry
59	1814	*Salmonella* Gallinarum	poultry
60	1815	*Salmonella Typhi*	poultry
61	1816	*Salmonella Panama*	poultry
62	6.5.1	*Typhimurium Salmonella*	poultry
63	6.5.2	*Typhimurium Salmonella*	poultry
64	1821	*Typhimurium Salmonella*	poultry
65	1800	*Salmonella Enteritidis*	human
66	1819	*Salmonella Enteritidis*	human
67	1667	*Salmonella Nigeria*	human
68	1820	*Typhimurium Salmonella*	human
69	1823	*Salmonella Dytona*	Animal
70	1284	*Typhimurium Salmonella*	Animal

Table 2. Combined disk test in this study

1	Cefapodoxim 30 µg Cefapodoxim 30 µg + 10 µg of Clavulanic acid
2	Cefotaxim 30 µg Cefotaxim 30 µg + 10 µg of Clavulanic acid
3	Ceftazidime 30 µg Ceftazidime 30 µg + 10 µg of Clavulanic acid
4	Cefepime 30 µg Cefepim 30 µg + 10 µg of Clavulanic acid

Table 3. Specifications of specific primers for blaTEM, blaSHV, blaCTX-M and blaOXA genes

	Target gene Primer sequence Product size Annealing Temp	References
1	blaTEM TEM-R 5´-ACG CTC AGT GGA ACG AAA AC -3 TEM-F 5' -ATT CTT GAA GAC GAA AGG GC -3´ ≈1200 57 o C	[9]
2	blaSHV SHV-R 5´-TTAGCGTTGCCAGTGCTCG -3´ SHV-F 5´-CACTCAAGGATGTATTGTG -3´ ≈950 60 o C	[10]
3	blaCTX-M CTX-M-R 5´-TGGGTAAAATAGGTGACCAGA -3´ CTX-M-F 5´-ATGTGCAGCACCAGTAAGGT -3´ ≈650 55 o C	[11]
4	blaOXA OXA-R 5-´CGACTTGATTGAAGGGTTGG-3´ OXA-F 5-´GGAGCAGCAACGATGTTACG-3´≈1353 51 o C	[12]

RESULTS

Strain serotype

The distribution of *salmonella* serotypes was determined in this study by determining the serum groups of isolates. Different types of serotypes that were present in this study included: *typhimurium* (24 %), *typhi* (1.4 %) *arizona* (3 %), *enteritidis* (22 %) *infancy* (7 %), *congo* (3 %), *newport (8 %), muenchen (1.4%), Dytona (1.4 %), Nigeria (1.4 %), Urbana (1.4 %), Victeraborg (1.4 %), Gallinarium (3 %), Panama (1.4 %), Dublin (1.4 %), and S II (1.4 %).*

Determination of antibiotic susceptibility

Determination of antibiotic resistance of different *salmonella* serotypes, isolated from 27 antibiotics showed that in this study, all 70 isolates were sensitive to gentamicin antibiotics, ciprofloxacin, ofloxacin, imipenem and enrofloxacin.

The highest resistance was observed for cefalexin antibiotics (96 %), cefazolin (96 %), cephalothin (65 %). There are 33 cases of Intermediate resistance to neomycin, 20 cases of amoxicillin antibiotics and 18 cases of cefalutin. Ceftriaxone (2 %) ureidopenicillin (3 %) had the least number of resistances, followed by ceftazidime, ceftoxime, ceftizoxime and nalidixic acid (4 %). 59 (84 %), with 3 or (3≥) antibiotic resistance, were presented as a multi-drug resistant drug. In this case, isolates of multiple resistance were observed in 98 % of MDR-resistant isolates against cefazolin and cefazolin. (Table 4).

Determination of ESBL producing strains

Among the 70 isolates studied in this study, the study of the differences in the growth zone diameter in Cefapodoxim, Cefotaxim and Ceftazidim and Cefepim with their clavulanic acid disks, showed that a total of 25 isolates (35 %) produced broad-spectrum beta-lactamases (ESBLs).

PCR reaction was performed on isolates of ESBL producing by combination phenotypic disk using specific primers to investigate the presence *of* blaCTX, blaTEM, *blaSHV* and blaOXA type plasmid genes. In total, it was found that 32 isolates (56 %) had at least one of the above genes.

In this case, 14 isolates carrying blaSHV gene, as well as 10 isolates carrying blaTEM gene, 17 isolates carrying the blaCTX-M gene and 17 isolates related to the blaOXA gene were identified. In addition, 1 simultaneous isolate has 3 genes (CTX-M, TEM, OXA) and one isolate had three SHV, OXA, and CTX-M genes, which is usually a rare phenomenon in *salmonella* (Figure 1).

DISCUSSION

The prevalence of resistance to antibiotics among *salmonella* is a major problem in the treatment of *salmonella* infections. The development of broad-spectrum cephalosporins in the early eighties has been instrumental in promoting a major tool in our struggle against bacterial resistance due to beta-lactams [13].

Chloramphenicol, ampicillin, trimethoprim, and sulfamethoxazole are the first antimicrobial drugs used in the treatment of *salmonella* infections, but over time the treatment encountered a problem with the development of drug resistance, in particular the multiple resistance to *salmonella typhi* and *non-typhi [14]*.

Salmonella appears to express a wide variety of ESBL types including TEM, SHV, PER, OXA and CTX-M.[15]. In this study, the presence of each of the studied genes in addition to poultry, animal and human specimens was shown to indicate the emergence of serotypes carrying these genes and the risk of antibiotic resistant serotypes being transmitted to humans. Therefore, it is necessary to conduct antibiotic susceptibility testing and continuous identification of these ESBL-producing serotypes in the community.

In the current study, typhimurium, paratyphi A, arizona, enteritidis, infancy, congo, newport, monchen, parathephi A, daytona, nigeria, urbana, victoria, gallinarum, panama, dublin, and SII were serotypes. Among these serotypes, typhimurium and enteritidis had the highest levels of isolates .In a study conducted in Spain in 2004 by Carraminana, the largest serotypes isolated from the herd were reported *by* newport, hadar, enteritidis, heidelberg, typhimurium and *virchow* [16]. In 2010, Ammar *et al.* isolated typhimurium and livingstone serotypes from poultry colloids [17]. Differences in isolated serotypes in different countries such as Iran can be the difference in the geographical areas tested.

Table 4. Determine the susceptibility of isolates to each of the antibiotics studied

Row	Antibiotics	Sensitive isolates (S)	Semi-sensitive isolates (I)	Resistant isolates (R)
1	Amoxicillin (AMX)	38(54%)	21(29%)	12(17%)
2	Amikacin (AN)	49(70%)	12(38%)	9(13%)
3	Ampicillin (AM)	67(95%)	3(5%)	0
4	Streptomycin (S)	59(83%)	7(11%)	4(6%)
5	Ofloxacin (OFX)	70 (100%)	0	0
6	Imipenem (IPM)	70 (100%)	0	0
7	Ureidopenicillin (PIP)	66(93%)	2(4%)	2(3%)
8	Tetracycline (TE)	46(65%)	12(18%)	12(17%)
9	Gentamicin (GM)	71(100%)	0	0
10	Cefazolin (CZ)	2(3%)	1(1%)	67(96%)

11	Cephalexin (CN)	3(4%)	0	67(96%)
12	Cefalotin (CF)	6(8%)	18(27%)	46(65%)
13	Ceftazidime (CAZ)	65(92%)	2(4%)	3(4%)
14	Cefaxime (CFM)	67(95%)	0	3(4%)
15	Ceftoxime (CTX)	66(93%)	4(7%)	0
16	Cefepime (FEP)	68(96%)	2(4%)	0
17	Ceftizoxime (CT)	63(89%)	4(7%)	3(4%)
18	Ciprofloxacin (CP)	70 (100%)	0	0
19	Furazolidone (FR)	56(79%)	5(8%)	9(13%)
20	kanamycin (K)	61(85%)	5(8%)	5(7%)
21	Chloramphenicol (C)	66(94%)	0	4(6%)
22	Co-trimoxazole (SXT)	63(89%)	0	7(10%)
23	Nalidixic acid (NA)	61(86%)	6(10%)	3(4%)
24	Neomycin (N)	33(47%)	33(47%)	4(6%)
25	Ceftriaxone (CRO)	66(94%)	3(5%)	1(1%)
26	Enrofloxacin (NFX)	70 (100%)	0	0
27	Clistin(Cl)	35(23%)	5(8%)	42(%61)

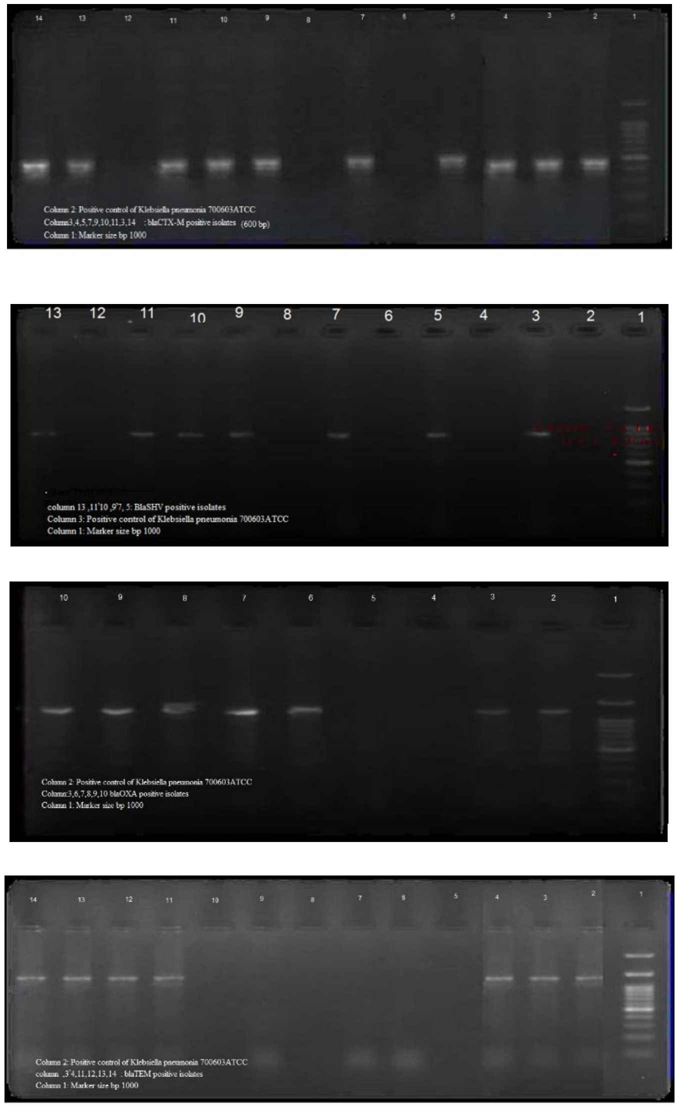

Figure 1. Electrophoresis components of PCR products for the genes of this study

Comparison of these results with the findings of this study showed that the isolates of this study were more susceptible to the antibiotics mentioned, so that the percentage of resistance to ampicillin (0 %), chloramphenicol (6 %), streptomycin (6 %), tetracycline (17 %) was reported to be less resistant. In a study conducted in Graziani *et al.* In Italy in 2008, the rate of resistance of isolated *salmonella* from humans and animals to resistance to tetracycline (73.6 %), sulfonamides (73.3 %), ampicillin (67.6 %), streptomycin (65.4 %) And chloramphenicol (32.3 %) [18].

The highest resistance to cephalexin antibiotic (96 %), cefazolin (96 %), cephalothin (65 %) was observed in the present study, which included beta-lactam antibiotics, and resistance to nalidixic acid (4 %). In 2009, Hamidian tested 129 isolates of *salmonella*, isolated from feces in patients with diarrhea in Tehran hospitals with the highest resistance to nalidixic acid (45.7 %), tetracycline (43.4 %), tritrimerum sulfamethoxyl (36.4 %), ampicillin (15.5 %) and chloramphenicol (14.7 %) [19]. The observation of resistance to first-generation cephalosporin antibiotics such as cephalothin, cefazolin, and cefalexin or older penicillin such as ampicillin was not far removed from the mind due to long-term and sustained use of them, or the appropriate effect of antibiotics; newer ones, such as third generation cephalosporins such as 4 % cefpodoxime and 4 % ceftazidime, are quite tangible due to the lack of resistance or a small amount of resistance to them. Increased resistance to antibiotics indicates the prevalence of plasmid resistance genes among different *salmonella* serotypes in humans, livestock and poultry.

In this study, 35 % of the isolates were producing ESBL. 14 % of the SHV gene and 10 % of the TEM gene were found after PCR, and three isolates (4.2 %) also had both genes. DE Gheldre in 2003, evaluated about 105 *salmonella* strains, of which 96 strains were positive in ESBL presence. The 71 strains of beta-lactamase-producing strains of the TEM-type ESBLs and 31 strains of SHV and both the TEM and SHV strains were found in 4 % of isolates [20].

During a research conducted in Japan in 2009, amongst the *salmonella* isolates, the highest resistance to spectinomycin (51.1 %), tetracycline (43 %), amoxicillin / clavulanic acid (4.40 %) and nalidixic acid (12.8 %) had the least resistance. All *salmonella* isolates were negative for SHV- , OXA- , CMY- and CTX- M- β-lactamase- encoding genes [21].

In this study, in order to investigate the antibiotic resistance of *salmonella enterica*, a producer of broad-spectrum β-lactamases (ESBLs), about 70 different strains of *salmonella* were studied, among which all 70 isolates, the gentamicin, ciprofloxacin and imipenem, and enrofloxacin were susceptible. The highest resistance to cephalexin antibiotics (96 %), cefazolin (96 %), cephalothin (65 %), and 69 isolates (98 %), were resistant to more than one antibiotic, of which 59 (84 %), with 3 or more resistances (3 ≥), antibiotics used in the rule were considered as isolates with multiple drug resistance. A total of 25 isolates, with combined disks (CTX / CV, CTX-CPD / CV, CPD-CPM / CV, CPM-CAZ / CV, CAZ) were produced by ESBLs. In 2005, Hasmon in the Netherlands, among human specimens, poultry and their products, 34 isolates of *salmonella*. All isolates were resistant to cefalotin, amoxicillin, except for 2 isolates, while others were resistant to cefurforum, cefuroxime and ceftazidime. Two isolates had complete resistance to amoxicillin/clavulanic acid, while one isolate showed an intermediate resistance to this compound. Except for 9 isolates, the remaining isolates were at least carrying one of the resistance genes of the type M-CTLBla, TEMbla and SHVbla, and at the same time some 2 genes of these 3 genes. However, the isolate carrying the three genes was not found simultaneously [22].

CONCLUSION

The results of the PCR reaction on ESBL producing isolates using specific primers; in order to investigate the presence of CTXbla, SHVbla, TEMbla and OXAbla, the presence of germ cell resistance genes in total indicated that 32 isolates (56 %), at least one of the above genes.

REFERENCES

[1]. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrobial agents chemother*, 2004. 48(1): 1-14.

[2]. Brenner F, *et al.* Salmonella nomenclature. *J Clinical Microbiol*, 2000. 38(7): 2465-67.

[3]. VT Nair, D., K. Venkitanarayanan, and A. Kollanoor Johny, Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Foods*, 2018. 7(10): 167.

[4]. Van den Bogaard, A, *et al.*, Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *J Antimicrobial Chemother*, 2001. 47(6): 763-71.

[5]. Perez, F., et al., The continuing challenge of ESBLs. *Current Opinion in Pharmacol*, 2007. 7(5): 459-69.

[6]. D'Andrea, G., Quercetin: a flavonol with multifaceted therapeutic applications? *Fitoterapia*, 2015. 106: 256-71.

[7]. Saima, S., *et al.*, Molecular investigation of antibiotic resistant bacterial strains isolated from

wastewater streams in Pakistan. 3 *Biotech,* 2020. 10(9): 1-11.

[8]. Harada, S., Y. Ishii, and K. Yamaguchi, Extended-spectrum β-lactamases: implications for the clinical laboratory and therapy. *Korean J Lab Med*, 2008. 28(6): 401-12.

[9]. Liebana, E., *et al*., Longitudinal farm study of extended-spectrum β-lactamase-mediated resistance. *J Clinical Microbiol*, 2006. 44(5): 1630-34.

[10]. Ali, T., *et al.*, ESBL-Producing *Escherichia coli* from cows suffering mastitis in China contain clinical class 1 integrons with CTX-M linked to IS CR1. *Frontiers in Microbiol*, 2016. 7: 1931.

[11]. Shahid, M., Citrobacter spp. simultaneously harboring bla CTX-M, bla TEM, bla SHV, bla ampC, and insertion sequences IS 26 and orf513: an evolutionary phenomenon of recent concern for antibiotic resistance. *J Clinical Microbiol,* 2010. 48(5): 1833-38.

[12]. Wecker, P., *et al*., Transcriptional response of the model planctomycete Rhodopirellula baltica SH1T to changing environmental conditions. *BMC Genomics*, 2009. 10(1): 1-16.

[13]. Steward, C.D., *et al.*, Characterization of clinical isolates of Klebsiella pneumoniae from 19 laboratories using the national committee for clinical laboratory standards extended-spectrum β-lactamase detection methods. *J Clinical Microbiol,* 2001. 39(8): 2864-72.

[14]. Ahmed, A., Y. Ishida, and T. Shimamoto, Molecular characterization of antimicrobial resistance in *salmonella* isolated from animals in Japan. *J Applied Microbiol,* 2009. 106(2): 402-409.

[15]. Baraniak, A., *et al.*, Countrywide spread of CTX-M-3 extended-spectrum β-lactamase-producing microorganisms of the family enterobacteriaceae in Poland. *Antimicrobial Agents Chemother,* 2002. 46(1): 151-59.

[16]. Carramiñana JJ, *et al.*, High prevalence of multiple resistance to antibiotics in *salmonella* serovars isolated from a poultry slaughterhouse in Spain. *Veterinary Microbiol*, 2004. 104(1-2): 133-39.

[17]. Ammar, A., *et al*., Survey of *salmonella* serovars in broilers and laying breeding reproducers in East of Algeria. *J Infection in*

Developing Countries, 2010. 4(02): 103-106.

[18]. Graziani, C., *et al.*, Antimicrobial resistance in *Salmonella* enterica serovar typhimurium from human and animal sources in Italy. *Veterinary Microbiol*, 2008. 128(3-4): 414-18.

[19]. Hamidian, M., *et al*., Emergence of extended-spectrum beta-lactamases in clinical isolates of *Salmonella* enterica in Tehran, I*ran. J Infect Dis*, 2009. 62(5): 368-71.

[20]. De Gheldre, Y., *et al.*, Evaluation of oxoid combination discs for detection of extended-spectrum β-lactamases. *J Antimicrobial Chemother*, 2003. 52(4): 591-97.

[21]. Ahmed, A.M., *et al.*, Genetic basis of multidrug resistance in *salmonella* enterica serovars and typhimurium isolated from diarrheic calves in Egypt. *Acta tropica*, 2009. 111(2): 144-49.

[22]. Hasman, H., *et al*., β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *salmonella* from poultry, poultry products and human patients in the Netherlands. *J Antimicrobial Chemother*, 2005. 56(1): 115-21.